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2. **Patent application number** *(The Patent Office will fill in this part)* — 9828603.2

23 DEC 1998

3. **Full name, address and postcode of the or of each applicant** *(underline all surnames)*

PFIZER LIMITED
SANDWICH
KENT
CT13 9NJ

Patents ADP number *(if you know it)* — 68926730001

If the applicant is a corporate body, give the country/state of its incorporation — UNITED KINGDOM

4. **Title of the invention** — ENZYME

5. **Name of your agent** *(if you have one)* — D YOUNG & CO

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

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D YOUNG & CO
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P5894.GB

ENZYME

The present invention relates to an enzyme. The present invention also relates to a nucleotide sequence encoding same.

In particular, the present invention relates to novel nucleic acid sequences encoding novel phosphodiesterase enzymes.

The present invention also relates to the use of the novel nucleic acid and amino acid sequences in the diagnosis and treatment of disease.

The present invention also relates to the use of the novel nucleic acid and amino acid sequences to evaluate and/or to screen for agents that can modulate phosphodiesterase activity.

The present invention further relates to genetically engineered host cells that comprise or express the novel nucleic acid and amino acid sequences to evaluate and/or to screen for agents that can modulate phosphodiesterase activity.

Cyclic nucleotide phosphodiesterases (PDEs) are a family of enzymes that catalyse the degradation of cyclic nucleotides. Cyclic nucleotides, particularly cAMP (i.e. cyclic adenosine 3',5'-monophosphate), are important intracellular second messengers. PDEs are one cellular component that regulates the concentration of cyclic nucleotides. In recent years, at least seven PDE enzymes (such as PDEI - PDEVII), as well as many subtypes of these enzymes, have been defined based on substrate affinity and cofactor requirements (Beavo JA and Reifsnyder DH, Trends Pharmacol. Sci. 11:150 [1990]; Beavo J, In: Cyclic Nucleotide Phosphodiesterases: Structure, Regulation and Drug Action., Beavo J and Housley MD (Eds.). Wiley : Chichester, pp. 3-15 [1990]).

In slightly more detail, examples of PDEs (i.e. cyclic nucleotide phosphodiesterases) include: PDEI which is a Ca^{2+}/Calmodulin-dependent PDE; PDEII which is a cGMP stimulated PDE; PDEIII which is a cGMP inhibited PDE; PDEIV which is a high affinity cAMP-specific PDE; and PDEV which is a cGMP specific PDE.

Each PDE family may contain two or more isoforms (i.e. there may be two or more PDE isoenzymes). By way of example, mammalian PDE IV, the homologue of the *Drosophila* Dunce gene (Chen CN *et al.*, Proc. Nat. Acad. Sci. (USA) 83:9313 [1986]), is known to have four isoforms in the rat (Swinnen JV *et al.*, Proc. Nat. Acad. Sci. (USA) 86:5325 [1989]). Human PDEs are also known to occur as isoforms and have splice variants. For example, the cloning of one human isoform of PDEIV from monocytes was reported in 1990 (Livi GP *et al.*, Mol. Cell. Bio., 10:2678 [1990]). By way of further example, other workers have independently cloned three splice variants of PDEIV, which are now designated hPDEIV-B1, hPDEIV-B2, and hPDEIV-B3.

Teachings on a further cyclic nucleotide phosphodiesterase - namely CN PCDE8 - can be found in WO-A-97/35989. According to WO-A-97/35989, CN PCDE8 has two isozymes - which were designated CN PCDE8A and CN PCDE8B. The term isozyme is sometimes referred to in the art as isoform.

According to WO-A-97/35989, many inhibitors of different PDEs have been identified and some have undergone clinical evaluation. For example, PDEIII inhibitors are being developed as antithrombotic agents, as antihypertensive agents and as cardiotonic agents useful in the treatment of congestive heart failure. Rolipram, a PDEIII inhibitor, has been used in the treatment of depression and other inhibitors of PDEIII are undergoing evaluation as anti-inflammatory agents. Rolipram has also been shown to inhibit lipopolysaccharide (LPS) induced TNF-alpha which has been shown to enhance HIV-1 replication *in vitro*. Therefore, rolipram may inhibit HIV-1 replication (Angel *et al* 1995 AIDS 9:1137-44). Additionally, based on its ability to suppress the production of TNF alpha and beta and interferon gamma, rolipram has been shown to be effective in the treatment of encephalomyelitis, the experimental animal model for multiple sclerosis

(Sommer *et al*, 1995 Nat Med 1:244-248) and may be effective in the treatment of tardive dyskinesia (Sasaki *et al*, 1995 Eur J Phamacol 282:71-76).

According to WO-A-97/35989, there are also non-specific PDE inhibitors such as theophylline, used in the treatment of bronchial asthma and other respiratory diseases, and pentoxifylline, used in the treatment of intermittent claudication and diabetes-induced peripheral vascular disease. Theophylline is thought to act on airway smooth muscle function as well as in an anti-inflammatory or immunomodulatory capacity in the treatment of respiratory diseases (Banner *et al* 1995 Respir J 8:996-1000) where it is thought to act by inhibiting both CN PDE cAMP and cGMP hydrolysis (Banner *et al* 1995 Monaldi Arch Chest Dis 50:286-292). Pentoxifylline, also known to block TNF-alpha production, may inhibit HIV-1 replication (Angel *et al supra*). A list of CN PDE inhibitors is given in Beavo 1995 *supra*.

It has been speculated that selective inhibitors of PDEs, in addition to their isozymes and their subtypes, will lead to more effective therapy with fewer side effects. For example, see the teachings in the reviews of Wieshaar RE *et al*, (J. Med. Chem., 28:537 [1985]), Giembycz MA (Biochem. Pharm., 43:2041 [1992]) and Lowe JA and Cheng JB (Drugs of the Future, 17:799-807 [1992]).

Thus, for some applications it is desirable to have a selective inhibition of an individual type of PDE. Hence, the cloning and expression of a novel PDE would greatly aid the discovery of selective inhibitors.

According to a first aspect of the present invention there is provided an amino acid sequence comprising the sequence presented as Formula I or a variant, homologue, fragment or derivative thereof.

Formula I

An amino acid sequence comprising any one or more of peptide sequences or amino acids Z1 - Z12, any of which peptide sequence or amino acid Z1 - Z12 may be separated from another of said peptide sequence or amino acid Z1 - Z12 by a suitable peptide sequence or amino acid residue;

wherein:

Z1 = MSCLMVERCGE
Z2 = LFE
Z3 = P
Z4 = Q
Z5 = KCVCMLGD
Z6 = RLRGQTGV
Z7 = AERRGSYPFIDFRLLN
Z8 = TT
Z9 = SGEIGTKKKVKRLLSFQRYFHASRLLRGIIPQAPLHLLDEDYLGQARHMLSKVG
Z10 = WDFDIFLFDRLTNGNSLVTLLCHLFN
Z11 = HGLIHHFKLDMVTLHRFLVMVQEDYH
Z12 =
NPYHNAVHAADVTQAMHCYLKEPKLASFLTPLDIMLGLLAAAAHDVDHPGVNQPFLIKTNHHLANLYQNMSVL
ENHHWRSTIGMLRESRLLAHLPKEMT

and

wherein the amino acid sequence is made up of more than 200 amino acid residues.

The amino acid sequence of the present invention is sometimes referered to as PDE_XIV.

For convenience, we now present a Table indicating the codes used for the amino acids.

AMINO ACID	THREE LETTER ABBREVIATION	ONE LETTER SYMBOL
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any residue	Xaa	X

Preferably the amino acid sequence is made up of more than about 230 amino acid residues.

Preferably the amino acid sequence is made up of more than about 250 amino acid residues.

Preferably the amino acid sequence is made up of more than about 260 amino acid residues.

Preferably the amino acid sequence is made up of at least 268 amino acid residues.

Preferably, the amino acid sequence of Formula I comprises at least one or more of Z7, Z9, Z10, Z11, and Z12, or analogues thereof.

The term "analogue" as used herein means a sequence having a sequence similar to that of Formula I but wherein non-detrimental amino acid substitutions or deletions have been made.

Preferably, the amino acid sequence of Formula I comprises at least each of Z7, Z9, Z10, Z11, and Z12, or analogues thereof.

Preferably, the amino acid sequence of Formula I comprises at least each of Z1, Z5, Z6, Z7, Z9, Z10, Z11, and Z12, or analogues thereof.

Preferably, the amino acid sequence of Formula I comprises at least each of Z1 - Z12, or analogues thereof.

A preferred example of an amino acid sequence comprising the sequence presented as Formula I is the amino acid sequence shown as Formula II or a variant, homologue, fragment or derivative thereof.

Formula II

Z1-X1-Z2-X2-Z3-X3-Z4-X4-Z5-X5-Z6-X6-Z7-X7-Z8-X8-Z9-X9-Z10-X10-Z11-X11-Z12

wherein

each of Z1 - Z12 is as defined above; and

each of X1 - X11 is independently selected from a suitable peptide sequence or amino acid.

A more preferred example of an amino acid sequence comprising the sequence presented as Formula I is the amino acid sequence shown as Formula III or a variant, homologue, fragment or derivative thereof.

Formula III

Z1-X1-Z2-X2-Z3-X3-Z4-X4-Z5-X5-Z6-X6-Z7-X7-Z8-X8-Z9-X9-Z10-X10-Z11-X11-Z12

wherein

each of Z1 - Z12 is as defined above; and

X1 = V or I
X2 = S or N
X3 = E or D
X4 = SV or NA
X5 = V or I
X6 = P or R
X7 = N or S
X8 = H or Y
X9 = T or M
X10 = S or T
X11 = GH or SQ

Preferred examples of an amino acid sequence comprising the sequence presented as Formula I include the amino acids shown as: SEQ ID No. 1 or SEQ ID No. 2 or a variant, homologue, fragment or derivative thereof

It is to be noted that references to Formula I and/or to any one or more of Formula II - Formula III herein also apply equally to any one or more of: SEQ ID No. 1 or SEQ ID No. 2.

Preferably references to Formula I and/or to any one or more of Formula II - Formula III herein mean any one or more of: SEQ ID No. 1 or SEQ ID No. 2.

According to a second aspect of the present invention there is provided an amino acid sequence comprising the sequence presented as Formula I.

According to a third aspect of the present invention there is provided a nucleotide sequence encoding the amino acid sequence of the present invention.

According to a fourth aspect of the present invention there is provided a nucleotide sequence comprising the sequence presented as SEQ ID No. 3 or SEQ ID No. 4 or a variant, homologue, fragment or derivative thereof.

According to a fifth aspect of the present invention there is provided a nucleotide sequence comprising the sequence presented as SEQ ID No. 1 or SEQ ID No. 4.

According to a sixth aspect of the present invention there is provided a nucleotide sequence that is capable of hybridising to the nucleotide sequence according to the present invention.

According to a seventh aspect of the present invention there is provided a nucleotide sequence that is capable of hybridising to the nucleotide sequence according to sixth aspect of the present invention.

According to an eighth aspect of the present invention there is provided a vector comprising the nucleotide sequence according to the present invention.

According to a ninth aspect of the present invention there is provided a host cell into which has been incorporated the nucleotide sequence according to the present invention.

According to a tenth aspect of the present invention there is provided an assay method for identifying an agent that can affect PDE_XIV activity or expression, the assay method comprising contacting an agent with an amino acid according to the present invention or a nucleotide sequence according to the present invention; and measuring the activity or

expression of PDE_XIV; wherein a difference between a) PDE activity or expression in the absence of the agent and b) PDE activity or expression in the presence of the agent is indicative that the agent can affect PDE_XIV activity or expression.

The assay can be used to screen for agents useful in the treatment of CNS disorders. The assay can be used to screen for agents useful in the treatment of cardiovascular disorders. The assay can be used to screen for agents useful in the treatment of gastrointestinal disorders. The assay can be used to screen for agents useful in the treatment of cancer. The assay can be used to screen for agents useful in the treatment of sexual dysfunction - such as male erectile dysfunction (MED) or female sexual dysfunction.

According to an eleventh aspect of the present invention there is provided a process comprising the steps of: (a) performing the assay according to the present invention; (b) identifying one or more agents that do affect PDE_XIV activity or expression; and (c) preparing a quantity of those one or more identified agents.

According to a twelfth aspect of the present invention there is provided a method of affecting *in vivo* PDE_XIV activity or expression with an agent; wherein the agent is capable of affecting PDE_XIV activity or expression in an *in vitro* assay method; wherein the *in vitro* assay method is the assay method of the present invention.

According to a thirteenth aspect of the present invention there is provided the use of an agent in the preparation of a pharmaceutical composition for the treatment of a disease or condition associated with PDE_XIV, the agent is capable of having an effect on the activity or expression of PDE when assayed *in vitro* by the assay method of the present invention.

According to a fourteenth aspect of the present invention there is provided an enzyme capable of having an immunological reaction with an antibody raised against PDE_XIV.

According to a fifteenth aspect of the present invention there is provided a nucleotide sequence coding for a PDE, wherein the nucleotide sequence is obtainable from NCIMB 40995 or NCIMB 40996.

According to a sixteenth aspect of the present invention there is provided a PDE wherein the PDE is expressable from a nucleotide sequence obtainable from NCIMB 40995 or NCIMB 40996.

According to a seventeenth aspect of the present invention there is provided the use of an agent which has an effect on the activity of PDE_XIV or the expression thereof in the preparation of a pharmaceutical composition for the treatment of a disease or condition associated with PDE_XIV.

According to a further aspect of the present invention there is provided

A nucleotide sequence selected from:

(a) the nucleotide sequence presented as SEQ ID No. 3 or 4, or a variant, homologue, derivative or fragment thereof;

(b) the nucleotide sequence set out in SEQ ID No. 3 or 4, or the complement thereof;

(c) a nucleotide sequence capable of hybridising to the nucleotide sequence set out in SEQ ID No. 3 or 4, or a fragment thereof;

(d) a nucleotide sequence capable of hybridising to the complement of the nucleotide sequence set out in SEQ ID No. 3 or 4, or a fragment thereof; and

(e) a nucleotide sequence which is degenerate as a result of the genetic code to the nucleotides defined in (a), (b), (c) or (d).

Other aspects of the present invention include:

An isolated nucleotide sequence or protein sequence according to the present invention.

An assay method for identifying an agent that can affect the expression pattern of the nucleotide sequence of the present invention or the activity of the expression product thereof,

the assay method comprising

exposing the nucleotide sequence of the present invention or the expression product ("EP") thereof with an agent;

determining whether the agent modulates (such as affects the expression pattern or activity) the nucleotide sequence of the present invention or the expression product thereof.

An agent identified by the assay method of the present invention.

An agent identified by the assay method of the present invention, which agent has hitherto been unknown to have a PDE modulation effect in accordance with the present invention.

A process comprising the steps of:

(a) performing the assay of the present invention;

(b) identifying one or more agents that affect the expression pattern of the nucleotide sequence of the present invention or the activity of the expression product thereof;

(c) preparing a quantity of those one or more identified agents.

A process comprising the steps of:

(a) performing the assay according to the present invention;

(b) identifying one or more agents that affect the expression pattern of the nucleotide sequence of the present invention or the activity of the expression product thereof;

(c) preparing a pharmaceutical composition comprising one or more identified agents.

A process comprising the steps of:

(a) performing the assay according to the present invention;

(b) identifying one or more agents that affect the expression pattern of the nucleotide sequence of the present invention or the activity of the expression product thereof;

(c) modifying one or more identified agents to cause a different effect on the expression pattern of the nucleotide sequence of the present invention or the activity of the expression product thereof.

Use of an agent identified by an assay according to the present invention in the manufacture of a medicament which affects the expression pattern of the nucleotide sequence of the present invention or the activity of the expression product thereof.

A method of treating a target (which target can be a mammal, preferably a human), which method comprises delivering (such as administering or exposing) to the target an effective amount of an agent capable of modulating the expression pattern of the nucleotide sequence of the present invention or the activity of the expression product thereof.

A method of treating a target (which target can be a mammal, preferably a human), which method comprises delivering (such as administering or exposing) to the target an effective amount of an agent identified by an assay according to the present invention.

A method of inducing an immunoligical response in a subject, the method comprising administering to the subject the nucleotide sequence of the present invention or the expression product thereof.

Broad aspects of the present invention include:

A recombinant PDE_XIV enzyme.

A recombinant nucleotide sequence encoding a PDE_XIV enzyme.

Preferably the recombinant PDE_XIV enzyme and/or the recombinant nucleotide sequence of the present invention are a recombinant mammalian PDE_XIV enzyme and/or a recombinant mammalian nucleotide sequence.

In accordance with the present invention, the recombinant PDE_XIV enzyme has at least the structure presented as Formula I.

Thus the present invention relates to a novel PDE enzyme - which we have called PDE_XIV - and to a nucleotide sequence encoding same. The present invention also relates to the use of the novel nucleic acid and amino acid sequences in the diagnosis and treatment of disease. The present invention also relates to the use of the novel nucleic acid and amino acid sequences to evaluate and/or to screen for agents that can modulate phosphodiesterase activity. The present invention further relates to genetically engineered host cells that comprise or express the novel nucleic acid and amino acid sequences to evaluate and/or to screen for agents that can modulate phosphodiesterase activity.

The term "nucleotide sequence" as used herein refers to an oligonucleotide sequence or polynucleotide sequence, and variants, homologues, fragments and derivatives thereof (such as portions thereof). The nucleotide sequence may be DNA or RNA of genomic or synthetic or recombinant origin which may be double-stranded or single-stranded whether representing the sense or antisense strand.

Preferably, the term "nucleotide sequence" means DNA.

More preferably, the term "nucleotide sequence" means DNA prepared by use of recombinant DNA techniques (i.e. recombinant DNA).

In a preferred embodiment, the nucleotide sequence *per se* of the present invention does not cover the native nucleotide coding sequence according to the present invention in its natural environment when it is under the control of its native promoter which is also in its natural environment. For ease of reference, we shall call this preferred embodiment the "non-native nucleotide sequence".

As used herein "amino acid sequence" refers to peptide or protein sequences or portions thereof.

In a preferred embodiment, the amino acid sequence *per se* the present invention does not cover the native PDE_XIV according to the present invention when it is in its natural environment and when it has been expressed by its native nucleotide coding sequence which is also in its natural environment and when that nucleotide sequence is under the control of its native promoter which is also in its natural environment. For ease of reference, we shall call this preferred embodiment the "non-native amino acid sequence".

As used herein, the term "PDE_XIV" refers to a novel PDE which, up until now, has been uncharacterised. By way of example, we have identified a human PDE_XIV (sometimes referred to as HSPDE_XIV). In addition, we have identified a mouse PDE_XIV (sometimes referred to as MMPDE_XIV).

For convenience, and unless otherwise stated, reference to PDE_XIV will include reference to HSPDE_XIV and/or MMPDE_XIV.

PDE_XIV is believed to be present in, and obtainable from, a variety of organs (see the data in Figures 1 - 3; which are discussed in the Experimental Section). We also believe that PDE_XIV is available from a number of sources in addition to mouse and human - such as any one or more of rat and bovine, ovine, porcine, and equine sources.

Preferably, the present invention covers mammalian PDE_XIV which includes but is not limited to any of the above sources.

The PDE_XIV may be the same as the naturally occuring form - for this aspect, preferably the PDE_XIV is the non-native amino acid sequence - or is a variant, homologue, fragment or derivative thereof. In addition, or in the alternative, the PDE_XIV is isolated PDE_XIV and/or purified PDE_XIV. The PDE_XIV can be obtainable from or produced by any suitable source, whether natural or not, or it may be synthetic, semi-synthetic or recombinant.

The PDE_XIV coding sequence may be the same as the naturally occuring form - for this aspect, preferably the PDE_XIV coding sequence is the non-native nucleotide sequence - or is a variant, homologue, fragment or derivative thereof. In addition, or in the alternative, the PDE_XIV coding sequence is an isolated PDE_XIV coding sequence and/or a purified PDE_XIV coding sequence. The PDE_XIV coding sequence can be obtainable from or produced by any suitable source, whether natural or not, or it may be synthetic, semi-synthetic or recombinant.

As used herein "naturally occurring" refers to a PDE_XIV with an amino acid sequence found in nature.

As used herein "biologically active" refers to a PDE_XIV having structural, regulatory or biochemical functions (but not necessarily to the same degree) of the naturally occurring PDE_XIV. Specifically, a PDE_XIV of the present invention has the ability to hydrolyze a cyclic nucleotide.

As used herein, "immunological activity" is defined as the capability of the natural, recombinant or synthetic PDE_XIV or any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The term "derivative" as used herein includes chemical modification of a PDE_XIV. Illustrative of such modifications would be replacement of hydrogen by an alkyl, acyl, or amino group.

As used herein, the terms "isolated" and "purified" refer to molecules, either nucleic or amino acid sequences, that are removed from their natural environment and isolated or separated from at least one other component with which they are naturally associated.

The terms "variant", "homologue" or "fragment" in relation to the amino acid sequence for the enzyme of the present invention include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) amino acid from or to the sequence providing the resultant enzyme has PDE_XIV activity, preferably being at least as biologically active as the enzyme shown as SEQ ID No. 1. or SEQ ID No. 2. In particular, the term "homologue" covers homology with respect to structure and/or function. With respect to sequence homology, preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to the sequence shown as Formula I. More preferably there is at least 95%, more preferably at least 98%, homology to the sequence shown as Formula I. More preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to the sequence shown as SEQ ID No. 1. or SEQ ID No. 2. More preferably there is at least 95%, more preferably at least 98%, homology to the sequence shown as SEQ ID No. 1. or SEQ ID No. 2.

With respect to the types of amino acid substitutions that could be made, typically, modifications can be made that maintain the hydrophobicity/hydrophilicity of the amino acid sequence. Amino acid substitutions may be made, for example from 1, 2 or 3 to 10, 20 or 30 substitutions provided that the modified sequence retains the ability to act as a PDE enzyme in accordance with present invention. Amino acid substitutions may include the use of non-naturally occurring analogues, for example to increase blood plasma half-life.

Conservative substitutions may be made, for example according to the Table below.

Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar - uncharged	C S T M
		N Q
	Polar - charged	D E
		K R
AROMATIC		H F W Y

The terms "variant", "homologue" or "fragment" in relation to the nucleotide sequence coding for the preferred enzyme of the present invention include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the sequence providing the resultant nucleotide sequence codes for or is capable of coding for an enzyme having PDE_XIV activity, preferably being at least as biologically active as the enzyme encoded by the sequences shown as SEQ ID No. 3 or SEQ ID No. 4. In particular, the term "homologue" covers homology with respect to structure and/or function providing the resultant nucleotide sequence codes for or is capable of coding for an enzyme having PDE_XIV activity. With respect to sequence homology, preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to a nucleotide sequence coding for the amino acid sequence shown as Formula I. More preferably there is at least 95%, more preferably at least 98 homology to a nucleotide sequence coding for the amino acid sequence shown as Formula I. Preferably, with respect to sequence homology, preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to the sequence shown as SEQ ID No. 3 or SEQ ID No. 4. More preferably there is at least 95%, more preferably at least 98%, homology to the sequence shown as SEQ ID No. 3 or SEQ ID No. 4.

In particular, the term "homology" as used herein may be equated with the term "identity". Here, sequence homology can be determined by a simple "eyeball" comparison (i.e. a strict comparison) of any one or more of the sequences with another sequence to see if that other sequence has at least 75% identity to the sequence(s). Relative sequence homology (i.e. sequence identity) can be determined by commercially available computer programs that can calculate % homology between two or more sequences. A typical example of such a computer program is CLUSTAL.

Sequence homology (or identity) may even be determined using any suitable homology algorithm, using for example default parameters. Advantageously, the BLAST algorithm is employed, with parameters set to default values. The BLAST algorithm is described in detail at http://www.ncbi.nih.gov/BLAST/blast_help.html, which is incorporated herein by reference. The search parameters are defined as follows, and are advantageously set to the defined default parameters.

Advantageously, "substantial homology" when assessed by BLAST equates to sequences which match with an EXPECT value of at least about 7, preferably at least about 9 and most preferably 10 or more. The default threshold for EXPECT in BLAST searching is usually 10.

BLAST (Basic Local Alignment Search Tool) is the heuristic search algorithm employed by the programs blastp, blastn, blastx, tblastn, and tblastx; these programs ascribe significance to their findings using the statistical methods of Karlin and Altschul (see http://www.ncbi.nih.gov/BLAST/blast_help.html) with a few enhancements. The BLAST programs were tailored for sequence similarity searching, for example to identify homologues to a query sequence. The programs are not generally useful for motif-style searching. For a discussion of basic issues in similarity searching of sequence databases, see Altschul *et al* (1994) Nature Genetics 6:119-129.

The five BLAST programs available at http://www.ncbi.nlm.nih.gov perform the following tasks:

blastp - compares an amino acid query sequence against a protein sequence database;

blastn - compares a nucleotide query sequence against a nucleotide sequence database;

blastx - compares the six-frame conceptual translation products of a nucleotide query sequence (both strands) against a protein sequence database;

tblastn - compares a protein query sequence against a nucleotide sequence database dynamically translated in all six reading frames (both strands).

tblastx - compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database.

BLAST uses the following search parameters:

HISTOGRAM - Display a histogram of scores for each search; default is yes. (See parameter H in the BLAST Manual).

DESCRIPTIONS - Restricts the number of short descriptions of matching sequences reported to the number specified; default limit is 100 descriptions. (See parameter V in the manual page). See also EXPECT and CUTOFF.

ALIGNMENTS - Restricts database sequences to the number specified for which high-scoring segment pairs (HSPs) are reported; the default limit is 50. If more database sequences than this happen to satisfy the statistical significance threshold for reporting (see EXPECT and CUTOFF below), only the matches ascribed the greatest statistical significance are reported. (See parameter B in the BLAST Manual).

EXPECT - The statistical significance threshold for reporting matches against database sequences; the default value is 10, such that 10 matches are expected to be found merely by chance, according to the stochastic model of Karlin and Altschul (1990). If the statistical significance ascribed to a match is greater than the EXPECT threshold, the

match will not be reported. Lower EXPECT thresholds are more stringent, leading to fewer chance matches being reported. Fractional values are acceptable. (See parameter E in the BLAST Manual).

CUTOFF - Cutoff score for reporting high-scoring segment pairs. The default value is calculated from the EXPECT value (see above). HSPs are reported for a database sequence only if the statistical significance ascribed to them is at least as high as would be ascribed to a lone HSP having a score equal to the CUTOFF value. Higher CUTOFF values are more stringent, leading to fewer chance matches being reported. (See parameter S in the BLAST Manual). Typically, significance thresholds can be more intuitively managed using EXPECT.

MATRIX - Specify an alternate scoring matrix for BLASTP, BLASTX, TBLASTN and TBLASTX. The default matrix is BLOSUM62 (Henikoff & Henikoff, 1992). The valid alternative choices include: PAM40, PAM120, PAM250 and IDENTITY. No alternate scoring matrices are available for BLASTN; specifying the MATRIX directive in BLASTN requests returns an error response.

STRAND - Restrict a TBLASTN search to just the top or bottom strand of the database sequences; or restrict a BLASTN, BLASTX or TBLASTX search to just reading frames on the top or bottom strand of the query sequence.

FILTER - Mask off segments of the query sequence that have low compositional complexity, as determined by the SEG program of Wootton & Federhen (1993) Computers and Chemistry 17:149-163, or segments consisting of short-periodicity internal repeats, as determined by the XNU program of Claverie & States (1993) Computers and Chemistry 17:191-201, or, for BLASTN, by the DUST program of Tatusov and Lipman (see http://www.ncbi.nlm.nih.gov). Filtering can eliminate statistically significant but biologically uninteresting reports from the blast output (e.g., hits against common acidic-, basic- or proline-rich regions), leaving the more biologically interesting regions of the query sequence available for specific matching against database sequences.

Low complexity sequence found by a filter program is substituted using the letter "N" in nucleotide sequence (e.g., "NNNNNNNNNNNNN") and the letter "X" in protein sequences (e.g., "XXXXXXXXX").

Filtering is only applied to the query sequence (or its translation products), not to database sequences. Default filtering is DUST for BLASTN, SEG for other programs.

It is not unusual for nothing at all to be masked by SEG, XNU, or both, when applied to sequences in SWISS-PROT, so filtering should not be expected to always yield an effect. Furthermore, in some cases, sequences are masked in their entirety, indicating that the statistical significance of any matches reported against the unfiltered query sequence should be suspect.

NCBI-gi - Causes NCBI gi identifiers to be shown in the output, in addition to the accession and/or locus name.

Most preferably, sequence comparisons are conducted using the simple BLAST search algorithm provided at http://www.ncbi.nlm.nih.gov/BLAST.

Other computer program methods to determine identify and similarity between the two sequences include but are not limited to the GCG program package (Devereux *et al* 1984 Nucleic Acids Research 12: 387) and FASTA (Atschul *et al* 1990 J Molec Biol 403-410).

Should Gap Penalties be used when determining sequence identity, then preferably the following parameters are used:

FOR BLAST	
GAP OPEN	0
GAP EXTENSION	0

FOR CLUSTAL	DNA	PROTEIN	
WORD SIZE	2	1	K triple
GAP PENALTY	10	10	
GAP EXTENSION	0.1	0.1	

As used herein, the terms "variant", "homologue", "fragment" and "deriavtive" embrace allelic variations of the sequences.

The term "variant" also encompasses sequences that are complementary to sequences that are capable of hydridising to the nucleotide sequences presented herein.

Preferably, the term "variant" encompasses sequences that are complementary to sequences that are capable of hydridising under stringent conditions (e.g. 65°C and 0.1xSSC {1xSSC = 0.15 M NaCl, 0.015 Na_3 citrate pH 7.0}) to the nucleotide sequences presented herein.

The present invention also covers nucleotide sequences that can hybridise to the nucleotide sequences of the present invention (including complementary sequences of those presented herein). In a preferred aspect, the present invention covers nucleotide sequences that can hybridise to the nucleotide sequence of the present invention under stringent conditions (e.g. 65°C and 0.1xSSC).

The present invention also relates to nucleotide sequences that are complementary to sequences that can hybridise to the nucleotide sequences of the present invention (including complementary sequences of those presented herein).

The term "vector" includes expression vectors and transformation vectors.

The term "expression vector" means a construct capable of *in vivo* or *in vitro* expression.

The term "transformation vector" means a construct capable of being transferred from one species to another.

Thus, the present invention relates to a novel PDE and to a novel nucleic acid sequence encoding same. We have designated this novel PDE as PDE_XIV.

Either or both of the nucleotide sequence coding for PDE_XIV or the enzyme PDE_XIV itself may be used to screen for agents that can affect PDE_XIV activity. In particular, the nucleotide sequence coding for PDE_XIV or PDE_XIV itself may be used to screen for agents that can inhibit PDE_XIV activity. In addition, the nucleotide sequence coding for PDE_XIV or the enzyme PDE_XIV itself may be used to screen for agents that selectively affect PDE_XIV activity, such as selectively inhibit PDE_XIV activity.

Furthermore, the nucleotide sequence coding for PDE_XIV or a sequence that is complementary thereto may also be used in assays to detect the presence of PDE_XIV coding sequences in human cells. These assays would provide information regarding the tissue distribution of this enzyme and its biological relevance with respect to particular disease states.

The present invention also covers antibodies to PDE_XIV (including a derivative, fragment, homologue or variant thereof). The antibodies for PDE_XIV may be used in assays to detect the presence of PDE_XIV in human cells. These assays would provide information regarding the tissue distribution of this enzyme and its biological relevance with respect to particular disease states.

PDE_XIV is believed to be able to catalyse the conversion of cAMP to AMP. Thus, PDE_XIV and/or its coding sequence and/or a sequence capable of hybridising thereto is/are useful for testing the selectivity of drug candidates between different PDEs.

As indicated, the present invention relates to a DNA sequence (preferably a cDNA sequence) encoding PDE_XIV. The present invention also relates to DNA segments comprising the DNA sequence of SEQ ID No. 3 or SEQ ID No. 4 or allelic variations of such sequences.

The present invention also relates to polypeptides produced by expression in a host cell into which has been incorporated the foregoing DNA sequences or allelic variations thereof.

A highly preferred aspect of the present invention relates to a polypeptide comprising the amino acid sequence of Formula I (such as SEQ ID No. 1 or SEQ ID No. 2). For example, the present invention relates to an isolated polypeptide comprising the amino acid sequence of Formula I (such as SEQ ID No. 1 or SEQ ID No. 2).

The present invention also relates to DNA comprising the DNA sequence of SEQ ID No. 3 or SEQ ID No. 4 or an allelic variation thereof.

The present invention also relates to non-native DNA comprising the DNA sequence of SEQ ID No. 3 or SEQ ID No. 4 or an allelic variation thereof.

A highly preferred aspect of the present invention relates to recombinant DNA comprising the DNA sequence of SEQ ID No. 3 or SEQ ID No. 4 or an allelic variation thereof.

The present invention also relates to an assay method for detecting the presence of PDE_XIV in cells (such as human cells) comprising: (a) performing a reverse transcriptase-polymerase chain reaction on RNA (such as total RNA) from such cells using a pair of polymerase chain reaction primers that are specific for PDE_XIV, as determined from the DNA sequence of SEQ ID No. 3 or SEQ ID No. 4 or an allelic variation thereof; and (b) assaying the appearance of an appropriately sized PCR (polymerase chain reaction) fragment - such as by agarose gel electrophoresis.

The present invention also relates to a method of identifying agents (such as compounds, other substances or compositions comprising same) that affect (such as inhibit or otherwise modify) the activity of PDE_XIV and/or the expression thereof, the method comprising contacting PDE_XIV or the nucleotide sequence coding for same with the agent and then measuring the activity of PDE_XIV and/or the expression thereof.

The present invention also relates to a method of identifying agents (such as compounds, other substances or compositions comprising same) that selectively affect (such as inhibit or otherwise modify) the activity of PDE_XIV and/or the expression thereof, the method comprising contacting PDE_XIV or the nucleotide sequence coding for same with the agent and then measuring the activity of PDE_XIV and/or the expression thereof.

The present invention also relates to a method of identifying agents (such as compounds, other substances or compositions comprising same) that affect (such as inhibit or otherwise modify) the activity of PDE_XIV and/or the expression thereof, the method comprising measuring the activity of PDE_XIV and/or the expression thereof in the presence of the agent or after the addition of the agent in: (a) a cell line into which has been incorporated recombinant DNA comprising the DNA sequence of SEQ ID No. 3 or SEQ ID No. 4 or an allelic variation thereof, or (b) a cell population or cell line that naturally selectively expresses PDE_XIV. Preferably, the activity of PDE_XIV is determined by the assay method described above.

The present invention also relates to a method of identifying agents (such as compounds, other substances or compositions comprising same) that selectively affect (such as inhibit or otherwise modify) the activity of PDE_XIV and/or the expression thereof, the method comprising measuring the activity of PDE_XIV and/or the expression thereof in the presence of the agent or after the addition of the agent in: (a) a cell line into which has been incorporated recombinant DNA comprising the DNA sequence of SEQ ID No. 3 or SEQ ID No. 4 or an allelic variation thereof, or (b) a cell population or cell line that naturally selectively expresses PDE_XIV. Preferably, the activity of PDE_XIV is determined by the assay method described above.

The present invention also relates to nucleotide sequences that are capable of hybridising to all or part of SEQ ID No. 3 or SEQ ID No. 4 or an allelic variation thereof. These nucleotide sequences may be used in anti-sense techniques to modify PDE_XIV expression. Alternatively, these sequences (or portions thereof) can be used as a probe, or for amplifying all or part of such sequence when used as a polymerase chain reaction primer.

The present invention also provides a diagnostic composition for the detection of PDE_XIV polynucleotide sequences. The diagnostic composition may comprise the polynucleotide SEQ ID No. 3 or SEQ ID No. 4 or a variant, homologue, fragment or derivative thereof, or a sequence capable of hybridising to all or part of SEQ ID No. 3 or SEQ ID No. 4 or an allelic variation thereof.

The present invention also provides a method of transforming a host cell with a nucleotide sequence shown as SEQ ID No. 3 or SEQ ID No. 4 or a derivative, homologue, variant or fragment thereof.

The present invention also provides a method for producing a polypeptide having PDE_XIV activity, the method comprising the steps of a) transforming a host cell with a nucleotide sequence shown as SEQ ID No. 3 or SEQ ID No. 4 or a derivative, homologue, variant or fragment thereof; and b) culturing the transformed host cell under conditions suitable for the expression of said polypeptide.

The present invention also provides a method for producing a polypeptide having PDE_XIV activity, the method comprising the steps of a) culturing a host cell that has been transformed with a nucleotide sequence shown as SEQ ID No. 3 or SEQ ID No. 4 or a derivative, homologue, variant or fragment thereof under conditions suitable for the expression of said polypeptide; and b) recovering said polypeptide from the host cell culture.

The present invention also provides a method for producing a polypeptide having PDE_XIV activity, the method comprising the steps of a) transforming a host cell with a nucleotide sequence shown as SEQ ID No. 3 or SEQ ID No. 4 or a derivative, homologue, variant or fragment thereof; b) culturing the transformed host cell under conditions suitable for the expression of said polypeptide; and c) recovering said polypeptide from the host cell culture.

The present invention also provides a method of screening an agent for modulation (preferably for specific modulation) of PDE_XIV (or a derivative, homologue, variant or fragment thereof) activity or the expression of the nucleotide sequence coding for same (including a derivative, homologue, variant or fragment thereof), the method comprising the steps of: a) providing a candidate agent; b) combining PDE_XIV (or the derivative, homologue, variant or fragment thereof) or the nucleotide sequence coding for same (or the derivative, homologue, variant or fragment thereof) with the candidate agent for a time sufficient to allow modulation under suitable conditions; and c) detecting modulation of the candidate agent to PDE_XIV (or the derivative, homologue, variant or fragment thereof) or the nucleotide sequence coding for same (or the derivative, homologue, variant or fragment thereof) in order to ascertain if the candidate agent modulates PDE_XIV (or the derivative, homologue, variant or fragment thereof) activity or the expression of the nucleotide sequence coding for same (or the derivative, homologue, variant or fragment thereof).

The present invention also provides a method of screening an agent for specific binding affinity with PDE_XIV (or a derivative, homologue, variant or fragment thereof) or the nucleotide sequence coding for same (including a derivative, homologue, variant or fragment thereof), the method comprising the steps of: a) providing a candidate agent; b) combining PDE_XIV (or the derivative, homologue, variant or fragment thereof) or the nucleotide sequence coding for same (or the derivative, homologue, variant or fragment thereof) with the candidate agent for a time sufficient to allow binding under suitable conditions; and c) detecting binding of the candidate agent to PDE_XIV (or the derivative, homologue, variant or fragment thereof) or the nucleotide sequence coding for same (or the derivative, homologue, variant or fragment thereof) in order to ascertain

if the candidate agent binds to PDE_XIV (or the derivative, homologue, variant or fragment thereof) or the nucleotide sequence coding for same (or the derivative, homologue, variant or fragment thereof).

The present invention also provides a method of identifying an agent which is capable of modulating PDE_XIV, the method comprising the steps of: a) contacting the agent with PDE_XIV (or a derivative, homologue, variant or fragment thereof) or the nucleotide sequence coding for same (or the derivative, homologue, variant or fragment thereof), b) incubating the mixture of step a) with a cyclic nucleotide under conditions suitable for the hydrolysis of the cyclic nucleotide, c) measuring the amount of cyclic nucleotide hydrolysis, and d) comparing the amount of cyclic nucleotide hydrolysis of step c) with the amount of cyclic nucleotide hydrolysis obtained with PDE_XIV (or the derivative, homologue, variant or fragment thereof) or the nucleotide sequence coding for same (or the derivative, homologue, variant or fragment thereof) incubated without the compound, thereby determining whether the agent affects (such as stimulates or inhibits) cyclic nucleotide hydrolysis.

The agent of the present invention can be, for example, an organic compound or an inorganic compound. The agent can be, for example, a nucleotide sequence that is anti-sense to all or part of the sequence shown as SEQ ID No. 3 or SEQ ID No. 4.

The present invention also provides a pharmaceutical composition for treating an individual in need of same due to PDE_XIV activity, the composition comprising a therapeutically effective amount of an agent that affects (such as inhibits) said activity and a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

Thus, the present invention also covers pharmaceutical compositions comprising the agents of the present invention (an agent capable of modulating the expression pattern of the nucleotide sequence of the present invention or the activity of the expression product thereof and/or an agent identified by an assay according to the present invention). In this regard, and in particular for human therapy, even though the agents of the present invention can be administered alone, they will generally be administered in admixture

with a pharmaceutical carrier, excipient or diluent selected with regard to the intended route of administration and standard pharmaceutical practice.

By way of example, in the pharmaceutical compositions of the present invention, the agents of the present invention may be admixed with any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), or solubilising agent(s).

In general, a therapeutically effective daily oral or intravenous dose of the agents of the present invention is likely to range from 0.01 to 50 mg/kg body weight of the subject to be treated, preferably 0.1 to 20 mg/kg. The agents of the present invention may also be administered by intravenous infusion, at a dose which is likely to range from 0.001-10 mg/kg/hr.

Tablets or capsules of the agents may be administered singly or two or more at a time, as appropriate. It is also possible to administer the agents of the present invention in sustained release formulations.

Typically, the physician will determine the actual dosage which will be most suitable for an individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

Where appropriate, the pharmaceutical compositions can be administered by inhalation, in the form of a suppository or pessary, topically in the form of a lotion, solution, cream, ointment or dusting powder, by use of a skin patch, orally in the form of tablets containing excipients such as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs, solutions or suspensions containing flavouring or colouring agents, or they can be injected parenterally, for example intracavernosally, intravenously, intramuscularly or subcutaneously. For parenteral administration, the compositions may be best used in the form of a sterile aqueous solution which may contain other substances, for example enough salts or

monosaccharides to make the solution isotonic with blood. For buccal or sublingual administration the compositions may be administered in the form of tablets or lozenges which can be formulated in a conventional manner.

For some applications, preferably the compositions are administered orally in the form of tablets containing excipients such as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs, solutions or suspensions containing flavouring or colouring agents.

For parenteral administration, the compositions are best used in the form of a sterile aqueous solution which may contain other substances, for example enough salts or monosaccharides to make the solution isotonic with blood.

For buccal or sublingual administration the compositions may be administered in the form of tablets or lozenges which can be formulated in a conventional manner.

For oral, parenteral, buccal and sublingual administration to subjects (such as patients), the daily dosage level of the agents of the present invention may typically be from 10 to 500 mg (in single or divided doses). Thus, and by way of example, tablets or capsules may contain from 5 to 100 mg of active agent for administration singly, or two or more at a time, as appropriate. As indicated above, the physician will determine the actual dosage which will be most suitable for an individual patient and it will vary with the age, weight and response of the particular patient. It is to be noted that whilst the above-mentioned dosages are exemplary of the average case there can, of course, be individual instances where higher or lower dosage ranges are merited and such dose ranges are within the scope of this invention.

Generally, in humans, oral administration of the agents of the present invention is the preferred route, being the most convenient and, for example in MED, avoiding the well-known disadvantages associated with intracavernosal (i.c.) administration. A preferred oral dosing regimen for a typical patient could be from 25 to 100 mg of agent when required. In circumstances where the recipient suffers from a swallowing disorder or

from impairment of drug absorption after oral administration, the drug may be administered parenterally, e.g. sublingually or buccally.

For veterinary use, the agent of the present invention is typically administered as a suitably acceptable formulation in accordance with normal veterinary practice and the veterinary surgeon will determine the dosing regimen and route of administration which will be most appropriate for a particular animal. However, as with human treatment, it may be possible to administer the agent alone for veterinary treatments.

Typically, the pharmaceutical compositions - which may be for human or animal usage - will comprise any one or more of a pharmaceutically acceptable diluent, carrier, excipient or adjuvant. The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. As indicated above, the pharmaceutical compositions may comprise as - or in addition to - the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s).

In some embodiments of the present invention, the pharmaceutical compositions will comprise one or more of: an agent that has been screened by an assay of the present invention; an agent that is capable of interacting with any one or more of SEQ ID No. 1, SEQ ID No 2, SEQ ID No. 3 or SEQ ID No. 4 including derivatives, fragments, homologues or variants thereof or sequences capable of hybridising to SEQ ID No. 3 or SEQ ID No. 4.

Thus the invention provides a pharmaceutical composition comprising an agent of the present invention (or even a pharmaceutically acceptable salt thereof, or a pharmaceutically acceptable solvate thereof) together with a pharmaceutically acceptable diluent, excipient or carrier.

The pharmaceutical composition could be for veterinary (i.e. animal) usuage or for human usuage.

The invention further provides an agent of the present invention (or even a pharmaceutically acceptable salt thereof, or a pharmaceutically acceptable solvate thereof) or a pharmaceutical composition containing any of the foregoing, for use as a medicament.

The present invention also provides the use of an agent to affect PDE_XIV activity (such as to inhibit, modulate or agonise) in the stratum of the brain.

The present invention also provides a method of treating an individual in need of same due to PDE_XIV activity comprising administering to said individual an effective amount of the pharmaceutical composition of the present invention.

Typically, a physician will determine the actual dosage which will be most suitable for an individual subject and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited.

Despite the fact that WO-A-97/35989 does not teach or suggest the presence of PDE_XIV, let alone the sequence for same or the nucleotide sequence coding for same, this document provides some useful background teachings on cyclic nucleotide phosphodiesterases and nucleotide sequences coding for same, as well as on their uses. Some of those teachings have been presented in some of the following description - but with reference to the cyclic nucleotide phosphodiesterase and nucleotide sequence coding for same of the present invention.

In accordance with the present invention, PDE_XIV polynucleotide sequences which encode PDE_XIV, fragments of the polypeptide, fusion proteins or functional equivalents thereof, may be used to generate recombinant DNA molecules that direct the expression of PDE_XIV in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence, may be used to clone and express PDE_XIV. As will be understood by those of skill in the art, it may be advantageous to

produce PDE-encoding nucleotide sequences possessing non-naturally occurring codons. Codons preferred by a particular prokaryotic or eukaryotic host (Murray E *et al* (1989) Nuc Acids Res 17:477-508) can be selected, for example, to increase the rate of PDE_XIV expression or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, than transcripts produced from naturally occurring sequence.

Also included within the scope of the present invention are polynucleotide sequences that are capable of hybridizing to the nucleotide sequence of SEQ ID No.3 or SEQ ID No. 4 under conditions of intermediate to maximal stringency. Hybridization conditions are based on the melting temperature (Tm) of the nucleic acid binding complex, as taught in Berger and Kimmel (1987, Guide to Molecular Cloning Techniques, Methods in Enzymology, Vol 152, Academic Press, San Diego CA), and confer a defined "stringency" as explained below.

Maximum stringency typically occurs at about Tm-5°C (5°C below the Tm of the probe); high stringency at about 5°C to 10°C below Tm; intermediate stringency at about 10°C to 20°C below Tm; and low stringency at about 20°C to 25°C below Tm. As will be understood by those of skill in the art, a maximum stringency hybridization can be used to identify or detect identical polynucleotide sequences while an intermediate (or low) stringency hybridization can be used to identify or detect similar or related polynucleotide sequences.

Exemplary nucleic acids can alternatively be characterised as those nucleotide sequences which encode a PDE_XIV protein and hybridise to the DNA sequences set forth as SEQ ID No. 3 or SEQ ID No. 4, or a selected fragment of said DNA sequence. Preferred are such sequences encoding PDE_XIV which hybridise under high-stringency conditions to the sequence of SEQ ID No. 3 or SEQ ID No. 4.

Stringency of hybridisation refers to conditions under which polynucleic acids hybrids are stable. Such conditions are evident to those of ordinary skill in the field. As known to those of skill in the art, the stability of hybrids is reflected in the melting temperature

(Tm) of the hybrid which decreases approximately 1 to 1.5°C with every 1% decrease in sequence homology. In general, the stability of a hybrid is a function of sodium ion concentration and temperature. Typically, the hybridisation reaction is performed under conditions of higher stringency, followed by washes of varying stringency.

As used herein, high stringency refers to conditions that permit hybridisation of only those nucleic acid sequences that form stable hybrids in 1 M Na+ at 65-68 °C. High stringency conditions can be provided, for example, by hybridisation in an aqueous solution containing 6x SSC, 5x Denhardt's, 1 % SDS (sodium dodecyl sulphate), 0.1 Na+ pyrophosphate and 0.1 mg/ml denatured salmon sperm DNA as non specific competitor. Following hybridisation, high stringency washing may be done in several steps, with a final wash (about 30 min) at the hybridisation temperature in 0.2 - 0.1x SSC, 0.1 % SDS.

Moderate stringency refers to conditions equivalent to hybridisation in the above described solution but at about 60-62°C. In that case the final wash is performed at the hybridisation temperature in 1x SSC, 0.1 % SDS.

Low stringency refers to conditions equivalent to hybridisation in the above described solution at about 50-52°C. In that case, the final wash is performed at the hybridisation temperature in 2x SSC, 0.1 % SDS.

It is understood that these conditions may be adapted and duplicated using a variety of buffers, e.g. formamide-based buffers, and temperatures. Denhardt's solution and SSC are well known to those of skill in the art as are other suitable hybridisation buffers (see, e.g. Sambrook, et al., eds. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York or Ausubel, et al., eds. (1990) Current Protocols in Molecular Biology, John Wiley & Sons, Inc.). Optimal hybridisation conditions have to be determined empirically, as the length and the GC content of the probe also play a role.

Advantageously, the invention moreover provides nucleic acid sequence which are capable of hybridising, under stringent conditions, to a fragment of SEQ. ID. No. 3 or SEQ ID No. 4. Preferably, the fragment is between 15 and 50 bases in length. Advantageously, it is about 25 bases in length.

The term "hybridization" as used herein shall include "the process by which a strand of nucleic acid joins with a complementary strand through base pairing" (Coombs J (1994) Dictionary of Biotechnology, Stockton Press, New York NY) as well as the process of amplification as carried out in polymerase chain reaction technologies as described in Dieffenbach CW and GS Dveksler (1995, PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview NY).

As used herein a "deletion" is defined as a change in either nucleotide or amino acid sequence in which one or more nucleotides or amino acid residues, respectively, are absent.

As used herein an "insertion" or "addition" is that change in a nucleotide or amino acid sequence which has resulted in the addition of one or more nucleotides or amino acid residues, respectively, as compared to the naturally occurring PDE.

As used herein "substitution" results from the replacement of one or more nucleotides or amino acids by different nucleotides or amino acids, respectively.

Altered PDE_XIV polynucleotide sequences which may be used in accordance with the invention include deletions, insertions or substitutions of different nucleotide residues resulting in a polynucleotide that encodes the same or a functionally equivalent PDE. The protein may also have deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent PDE. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the biological activity of PDE is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids

include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine, valine, glycine, alanine, asparagine, glutamine, serine, threonine, phenylalanine, and tyrosine.

Included within the scope of the present invention are alleles of PDE. As used herein, an "allele" or "allelic sequence" is an alternative form of PDE. Alleles result from a mutation, i.e., a change in the nucleic acid sequence, and generally produce altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene may have none, one or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to deletions, additions or substitutions of amino acids. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

The nucleotide sequences of the present invention may be engineered in order to alter a PDE coding sequence for a variety of reasons, including but not limited to, alterations which modify the cloning, processing and/or expression of the gene product. For example, mutations may be introduced using techniques which are well known in the art, e.g., site-directed mutagenesis to insert new restriction sites, to alter glycosylation patterns or to change codon preference.

In another embodiment of the invention, a PDE natural, modified or recombinant sequence may be ligated to a heterologous sequence to encode a fusion protein. For example, for screening of peptide libraries for inhibitors of PDE activity, it may be useful to encode a chimeric PDE protein expressing a heterologous epitope that is recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between a PDE sequence and the heterologous protein sequence, so that the PDE may be cleaved and purified away from the heterologous moiety.

In an alternative embodiment of the invention, the coding sequence of PDE could be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers MH *et al* (1980) Nuc Acids Res Symp Ser 215-23, Horn T *et al* (1980) Nuc

Acids Res Symp Ser 225-232). Alternatively, the protein itself could be produced using chemical methods to synthesize a PDE amino acid sequence, in whole or in part. For example, peptides can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography (e.g., Creighton (1983) Proteins Structures And Molecular Principles, WH Freeman and Co, New York NY). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; Creighton, *supra*).

Direct peptide synthesis can be performed using various solid-phase techniques (Roberge JY *et al* (1995) Science 269: 202-204) and automated synthesis may be achieved, for example, using the ABI 43 1 A Peptide Synthesizer (Perkin Elmer) in accordance with the instructions provided by the manufacturer. Additionally, the amino acid sequence of PDE, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with a sequence from other subunits, or any part thereof, to produce a variant polypeptide.

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression should be confirmed. For example, if the PDE coding sequence is inserted within a marker gene sequence, recombinant cells containing PDE coding regions can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a PDE coding sequence under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of PDE as well.

Alternatively, host cells which contain the coding sequence for PDE and express PDE coding regions may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridization and protein bioassay or immunoassay techniques which include membrane-based, solution-based, or chip-based technologies for the detection and/or quantification of the nucleic acid or protein.

The presence of the PDE polynucleotide coding sequence can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes, portions or fragments of the sequence presented as SEQ ID No. 3 or SEQ ID No. 4. Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers based on the PDE coding sequence to detect transformants containing PDE DNA or RNA. As used herein "oligonucleotides" or "oligomers" may refer to a nucleic acid sequence of at least about 10 nucleotides and as many as about 60 nucleotides, preferably about 15 to 30 nucleotides, and more preferably about 20-25 nucleotides which can be used as a probe or amplimer. Preferably, oligonucleotides are derived from the 3' region of the nucleotide sequence shown as SEQ ID No: 3 or SEQ ID No. 4.

A variety of protocols for detecting and measuring the expression of PDE polypeptide, using either polyclonal or monoclonal antibodies specific for the protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on PDE polypeptides is preferred, but a competitive binding assay may be employed. These and other assays are described, among other places, in Hampton R *et al* (1990, Serological Methods, A Laboratory Manual, APS Press, St Paul MN) and Maddox DE *et al* (1983, J Exp Med 15 8:121 1).

A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic and amino acid assays. Means for producing labelled hybridization or PCR probes for detecting PDE polynucleotide sequences include oligolabelling, nick translation, end-labelling or PCR amplification using a labelled nucleotide. Alternatively, the PDE coding sequence, or any portion of it, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3 or SP6 and labeled nucleotides.

A number of companies such as Pharmacia Biotech (Piscataway, NJ), Promega (Madison, WI), and US Biochemical Corp (Cleveland, OH) supply commercial kits and protocols for these procedures. Suitable reporter molecules or labels include those radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles and the like. Patents teaching the use of such labels include US Patents 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149 and 4,366,241. Also, recombinant immunoglobulins may be produced as shown in US Patent No. 4,816,567.

Host cells transformed with a PDE nucleotide coding sequence may be cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The protein produced by a recombinant cell may be secreted or may be contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing PDE coding sequences can be designed with signal sequences which direct secretion of PDE coding sequences through a particular prokaryotic or eukaryotic cell membrane. Other recombinant constructions may join PDE coding sequence to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins (Kroll *DJ et al* (1993) DNA Cell Biol 12:441-53, see also above discussion of vectors containing fusion proteins).

The PDE may also be expressed as a recombinant protein with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals (Porath J (1992) Protein Expr Purif 3 -.26328 1), protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle, WA). The inclusion of a cleavable linker sequence such as Factor XA or enterokinase (Invitrogen, San Diego, CA) between the purification domain and PDE is useful to facilitate purification.

Specific amino acid sequences of PDE_XIV are shown as SEQ ID No. 1 and SEQ ID No. 2. However, the present invention encompasses amino acid sequences encoding other members from the PDE_XIV family which would include amino acid sequences having at least 60% identity (more preferably at least 75% identity) to the amino acid sequence of SEQ ID Nos. 1 or 2. As indicated, suitable generic formulae for the PDE_XIV family in accordance with the present invention are presented as Formula I or any one of Formula II-Formula III.

In an embodiment of the present invention, PDE_XIV or a variant, homologue, fragment or derivative thereof and/or a cell line that expresses the PDE_XIV or variant, homologue, fragment or derivative thereof may be used to screen for antibodies, peptides, or other agent, such as organic or inorganic molecules, that act as modulators of phosphodiesterase activity or for the expression thereof, thereby identifying a therapeutic agent capable of modulating cyclic nucleotide levels. For example, anti-PDE_XIV antibodies capable of neutralizing the activity of PDE_XIV may be used to inhibit PDE_XIV hydrolysis of cyclic nucleotides, thereby increasing their levels. Alternatively, screening of peptide libraries or organic libraries made by combinatorial chemistry with recombinantly expressed PDE_XIV or a variant, homologue, fragment or derivative thereof or cell lines expressing PDE_XIV or a variant, homologue, fragment or derivative thereof may be useful for identification of therapeutic agents that function by modulating PDE_XIV hydrolysis of cyclic nucleotides. Synthetic compounds, natural products, and other sources of potentially biologically active materials can be screened in a number of ways deemed to be routine to those of skill in the art. For example, nucleotide sequences encoding the N-terminal region of PDE_XIV may be expressed in a cell line which can be used for screening of allosteric modulators, either agonists or antagonists, of PDE_XIV activity. Alternatively, nucleotide sequences encoding the conserved catalytic domain of PDE_XIV can be expressed in cell lines and used to screen for inhibitors of cyclic nucleotide hydrolysis.

The ability of a test agent to interfere with PDE_XIV activity or cyclic nucleotide hydrolysis may be determined by measuring PDE_XIV levels or cyclic nucleotide levels (as disclosed in Smith *et al* 1993 Appl. Biochem. Biotechnol. 41:189-218). There are

also commercially available immunoassay kits for the measurement of cAMP and cGMP (eg Amersham International, Arlington Heights, IL and DuPont, Boston, MA). The activity of PDE_XIV may also be monitored by measuring other responses such as phosphorylation or dephosphorylation of other proteins using conventional techniques developed for these purpose.

Accordingly, the present invention provides a method of identifying a compound which is capable of modulating the cyclic nucleotide phosphodiesterase activity of a PDE_XIV, or a variant, homologue, fragment or derivative thereof, comprising the steps of a) contacting the compound with a PDE_XIV, or a variant, homologue, fragment or derivative thereof; b) incubating the mixture of step a) with a cyclic nucleotide under conditions suitable for the hydrolysis of the cyclic nucleotide; c) measuring the amount of cyclic nucleotide hydrolysis; and d) comparing the amount of cyclic nucleotide hydrolysis of step c) with the amount of cyclic nucleotide hydrolysis obtained with the PDE_XIV, or a variant, homologue, fragment or derivative thereof, incubated without the compound, thereby determining whether the compound stimulates or inhibits cyclic nucleotide hydrolysis. In one embodiment of the method, the fragment may be from the N-terminal region of the PDE_XIV and provides a method to identify allosteric modulators of the PDE_XIV. In another embodiment of the present invention, the fragment may be from the carboxy terminal region of the PDE_XIV and provides a method to identify inhibitors of cyclic nucleotide hydrolysis.

Procedures well known in the art may be used for the production of antibodies to PDE_XIV polypeptides. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by a Fab expression library. Neutralizing antibodies, i.e., those which inhibit biological activity of PDE polypeptides, are especially preferred for diagnostics and therapeutics.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, etc. may be immunized by injection with PDE_XIV polypeptide or any portion, variant, homologue, fragment or derivative thereof or oligopeptide which retains immunogenic properties. Depending on the host species, various adjuvants may be used to increase

immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminium hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. BCG (*Bacilli Calmette-Guerin*) and *Corynebacterium parvum* are potentially useful human adjuvants which may be employed if purified PDE polypeptide is administered to immunologically compromised individuals for the purpose of stimulating systemic defence.

Monoclonal antibodies to PDE_XIV polypeptide may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique originally described by Koehler and Milstein (1975 Nature 256:495-497), the human B-cell hybridoma technique (Kosbor *et al* (1983) Immunol Today 4:72; Cote *et al* (1983) Proc Natl Acad Sci 80:2026-2030) and the EBV-hybridoma technique (Cole *et al* (1985) Monoclonal Antibodies and Cancer Therapy, Alan R Liss Inc, pp 77-96). In addition, techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used (Morrison *et al* (1984) Proc Natl Acad Sci 81:6851-6855; Neuberger *et al* (1984) Nature 312:604-608; Takeda *et al* (1985) Nature 314:452-454). Alternatively, techniques described for the production of single chain antibodies (US Patent No. 4,946,779) can be adapted to produce PDE_XIV specific single chain antibodies.

Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in Orlandi *et al* (1989, Proc Natl Acad Sci 86: 3833-3837), and Winter G and Milstein C (1991; Nature 349:293-299).

Antibody fragments which contain specific binding sites for PDE_XIV may also be generated. For example, such fragments include, but are not limited to, the $F(ab')_2$ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$

fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse WD *et al* (1989) Science 256:1275-128 1).

PDE_XIV-specific antibodies are useful for the diagnosis of conditions and diseases associated with expression of PDE_XIV polypeptide. A variety of protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the formation of complexes between PDE_XIV polypeptides and its specific antibody (or similar PDE_XIV-binding molecule) and the measurement of complex formation. A two-site, monoclonal based immunoassay utilizing monoclonal antibodies reactive to two noninterfering epitopes on a specific PDE_XIV protein is preferred, but a competitive binding assay may also be employed. These assays are described in Maddox DE *et al* (1983, J Exp Med 158:121 1).

Anti-PDE_XIV antibodies are useful for the diagnosis of inflammation, conditions associated with proliferation of hematopoietic cells and HIV infection or other disorders or diseases characterized by abnormal expression of a PDE_XIV. Diagnostic assays for a PDE_XIV include methods utilizing the antibody and a label to detect a PDE_XIV polypeptide in human body fluids, cells, tissues or sections or extracts of such tissues. The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, the polypeptides and antibodies will be labeled by joining them, either covalently or noncovalently, with a reporter molecule. A wide variety of reporter molecules are known to those of skill in the art.

A variety of protocols for measuring a PDE_XIV polypeptide, using either polyclonal or monoclonal antibodies specific for the respective protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on a PDE_XIV polypeptide is preferred, but a competitive binding assay may be employed. These

assays are described, among other places, in Maddox, DE *et al* (1983, J Exp Med 158:1211).

In order to provide a basis for the diagnosis of disease, normal or standard values from a PDE_XIV polypeptide expression must be established. This is accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with antibody to a PDE_XIV polypeptide under conditions suitable for complex formation which are well known in the art. The amount of standard complex formation may be quantified by comparing it to a dilution series of positive controls where a known amount of antibody is combined with known concentrations of a purified PDE_XIV polypeptide. Then, standard values obtained from normal samples may be compared with values obtained from samples from subjects potentially affected by a disorder or disease related to a PDE_XIV polypeptide expression. Deviation between standard and subject values establishes the presence of the disease state.

A PDE_XIV polypeptide, its immunogenic fragments or oligopeptides thereof can be used for screening therapeutic compounds in any of a variety of drug screening techniques. The polypeptide employed in such a test may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The abolition of activity or the formation of binding complexes between a PDE_XIV polypeptide and the agent being tested may be measured.

Accordingly, the present invention provides a method for screening one or a plurality of compounds for modulation (preferably specific modulation, such as specific binding affinity) of PDE_XIV or the expression thereof, or a portion thereof or variant, homologue, fragment or derivative thereof, comprising providing one or a plurality of compounds; combining a PDE_XIV or a nucleotide sequence coding for same or a portion thereof or variant, homologue, fragment or derivative thereof with the or each of a plurality of compounds for a time sufficient to allow modulation under suitable conditions; and detecting binding of a PDE_XIV, or portion thereof or variant, homologue, fragment or derivative thereof, to each of the plurality of compounds, thereby identifying the compound or compounds which modulate a PDE_XIV or a

nucleotide sequence coding for same. In such an assay, the plurality of compounds may be produced by combinatorial chemistry techniques known to those of skill in the art.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the PDE_XIV polypeptides and is based upon the method described in detail in Geysen, European Patent Application 84/03564, published on September 13, 1984. In summary, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with PDE_XIV fragments and washed. A bound PDE_XIV is then detected - such as by appropriately adapting methods well known in the art. A purified PDE_XIV can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding a PDE_XIV specifically compete with a test compound for binding a PDE_XIV. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with a PDE_XIV.

A PDE_XIV polynucleotide, or any part thereof, may provide the basis for a diagnostic and/or a therapeutic compound. For diagnostic purposes, PDE_XIV polynucleotide sequences may be used to detect and quantitate gene expression in conditions, disorders or diseases in which PDE_XIV activity may be implicated, for example, in CNS disorder, cardiovascular disorder, GI disorders, cancer, male erectile dysfunction.

Included in the scope of the invention are oligonucleotide sequences, antisense RNA and DNA molecules and ribozymes, which function to destabilize PDE_XIV mRNA or inhibit translation of a PDE_XIV. Such nucleotide sequences may be used in conditions where it would be preferable to increase cyclic nucleotide levels, such as in inflammation.

Another aspect of the subject invention is to provide for nucleic acid hybridization or PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding PDE coding region or closely related molecules, such as alleles. The specificity of the probe, i.e., whether it is derived from a highly conserved, conserved or non-conserved region or domain, and the stringency of the hybridization or amplification (high, intermediate or low) will determine whether the probe identifies only naturally occurring PDE coding sequence, or related sequences. Probes for the detection of related nucleic acid sequences are selected from conserved or highly conserved nucleotide regions of cyclic nucleotide PDE family members, such as the 3' region, and such probes may be used in a pool of degenerate probes. For the detection of identical nucleic acid sequences, or where maximum specificity is desired, nucleic acid probes are selected from the non-conserved nucleotide regions or unique regions of PDE polynucleotides. As used herein, the term "non-conserved nucleotide region" refers to a nucleotide region that is unique to the PDE coding sequence disclosed herein and does not occur in related family members, such as known cyclic nucleotide PDEs.

A PDE_XIV encoding polynucleotide sequence may be used for the diagnosis of diseases resulting from expression of PDE_XIV. For example, polynucleotide sequences encoding PDE_XIV may be used in hybridization or PCR assays of tissues from biopsies or autopsies or biological fluids, such as serum, synovial fluid or tumor biopsy, to detect abnormalities in PDE_XIV expression. The form of such qualitative or quantitative methods may include Southern or northern analysis, dot blot or other membrane-based technologies; PCR technologies; dip stick, pin or chip technologies; and ELISA or other multiple sample formal technologies. All of these techniques are well known in the art and are in fact the basis of many commercially available diagnostic kits.

Such assays may be tailored to evaluate the efficacy of a particular therapeutic treatment regime and may be used in animal studies, in clinical trials, or in monitoring the treatment of an individual patient. In order to provide a basis for the diagnosis of disease, a normal or standard profile for PDE expression must be established. This is accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with PDE_XIV or a portion thereof, under conditions suitable

for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained for normal subjects with a dilution series of positive controls run in the same experiment where a known amount of purified PDE_XIV is used. Standard values obtained from normal samples may be compared with values obtained from samples from subjects potentially affected by a disorder or disease related to expression of the PDE coding sequence. Deviation between standard and subject values establishes the presence of the disease state. If disease is established, an existing therapeutic agent is administered, and treatment profile or values may be generated. Finally, the assay may be repeated on a regular basis to evaluate whether the values progress toward or return to the normal or standard pattern. Successive treatment profiles may be used to show the efficacy of treatment over a period of several days or several months.

PCR as described in US Patent Nos. 4,683,195; 4,800,195; and 4,965,188 provides additional uses for oligonucleotides based upon the PDE_XIV sequence. Such oligomers are generally chemically synthesized, but they may be generated enzymatically or produced from a recombinant source. Oligomers generally comprise two nucleotide sequences, one with sense orientation (5'->3') and one with antisense (3'<-5') employed under optimized conditions for identification of a specific gene or condition. The same two oligomers, nested sets of oligomers, or even a degenerate pool of oligomcrs may be employed under less stringent conditions for detection and/or quantitation of closely related DNA or RNA sequences.

Additional methods to quantitate the expression of a particular molecule include radiolabeling (Melby PC *et al* 1993 J Immunol Methods 159:235-44) or biotinylating (Duplaa C *et al* 1993 Anal Biochem 229-36) nucleotides, coamplification of a control nucleic acid, and standard curves onto which the experimental results are interpolated. Quantitation of multiple samples may be speeded up by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or calorimetric response gives rapid quantitation.

A PDE_XIV antisense molecule may provide the basis for treatment of various abnormal conditions related to, for example, increased PDE_XIV activity - such as CNS disorder, cardiovascular disorder, GI disorders, cancer, male erectile dysfunction.

Expression vectors derived from retroviruses, adenovirus, herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of recombinant PDE_XIV sense or antisense molecules to the targeted cell population. Methods which are well known to those skilled in the art can be used to construct recombinant vectors containing PDE_XIV. Alternatively, recombinant PDE_XIV can be delivered to target cells in liposomes.

The full length cDNA sequence and/or its regulatory elements enable researchers to use PDE_XIV as a tool in sense (Youssoufian H and HF Lodish 1993 Mol Cell Biol 13:98-104) or antisense (Eguchi *et al* (1991) Annu Rev Biochem 60:631-652) investigations of gene function. Oligonucleotides, designed from the cDNA or control sequences obtained from the genomic DNA can be used *in vitro* or *in vivo* to inhibit expression. Such technology is now well known in the art, and sense or antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions. Appropriate oligonucleotides, which can be 20 nucleotides in length, may be used to isolate PDE_XIV sequences or closely related molecules from human libraries.

Additionally, PDE_XIV expression can be modulated by transfecting a cell or tissue with expression vectors which express high levels of a PDE_XIV fragment in conditions where it would be preferable to block phosphodiesterase activity thereby increasing cyclic nucleotide levels. Such constructs can flood cells with untranslatable sense or antisense sequences. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until all copies of the vector are disabled by endogenous nucleases. Such transient expression may last for a month or more with a non-replicating vector and even longer if appropriate replication elements are part of the vector system.

Modifications of gene expression can be obtained by designing antisense sequences to the control regions of the PDE gene, such as the promoters, enhancers, and introns.

Oligonucleotides derived from the transcription initiation site, e.g., between -10 and +10 regions of the leader sequence, are preferred. Antisense RNA and DNA molecules may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes. Similarly, inhibition can be achieved using Hogeboom base-pairing methodology, also known as "triple helix" base pairing. Triple helix pairing compromises the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by a endonucleolytic cleavage. Within the scope of the invention are engineered hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of PDE RNA sequences.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for secondary structural features which may render the oligonucleotide sequence inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Both antisense RNA and DNA molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of RNA molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* or *in vivo* transcription of DNA sequences encoding the antisense RNA

molecule. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly can be introduced into cell lines, cells or tissues.

DNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences of the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule.

Methods for introducing vectors into cells or tissue include those methods discussed herein. In addition, several of these transformation or transfection methods are equally suitable for *ex vivo* therapy,

The nucleic acid sequence for PDE_XIV can also be used to generate hybridization probes as previously described, for mapping the endogenous genomic sequence. The sequence may be mapped to a particular chromosome or to a specific region of the chromosome using well known techniques. These include *in situ* hybridization to chromosomal spreads (Verma *et al* (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York City), flow-sorted chromosomal preparations, or artificial chromosome constructions such as YACs, bacterial artificial chromosomes (BACs), bacterial PI constructions or single chromosome cDNA libraries.

In situ hybridization of chromosomal preparations and physical mapping techniques such as linkage analysis using established chromosomal markers are invaluable in extending genetic maps. Examples of genetic maps can be found in Science (1995; 270:410f and 1994; 265:1981f). Often the placement of a gene on the chromosome of another mammalian species may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms, or parts thereof, by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once a disease or syndrome, such as ataxia telangiectasia

(AT), has been crudely localized by genetic linkage to a particular genomic region, for example, AT to 11q22-23 (Gatti *et al* (1988) Nature 336:577-580), any sequences mapping to that area may represent associated or regulatory genes for further investigation. The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc between normal, carrier or affected individuals.

Genomic sequences are also of utility in the context of drug discovery. It may be valuable to inhibit the mRNA transcription of a particular isoform rather than to inhibit its translated protein. This is particularly true with PDE_XIV, since the different splice variants may be transcribed from different promoters. There is precedent for multiple promoters directing the transcription of a mouse brain 2',3'-cyclic-nucleotide 3' phosphodiesterase (Kurihara T *et al.*, Biochem. Biophys. Res. Comm. 170:1074 [1990]).

Another utility of the invention is that the DNA sequences, once known, give the information that may be needed to design assays to specifically detect isoenzymes or splice variants. Isozyme-specific PCR primer pairs are but one example of an assay that depends completely on the knowledge of the specific DNA sequence of the isozyme or splice variant. Such an assay allows detection of mRNA for the isozyme to access the tissue distribution and biological relevance of each isozyme to a particular disease state. It also allows identification of cell lines that may naturally express only one isozyme - a discovery that might obviate the need to express recombinant genes. If specific PDE_XIV isozymes are shown to associated with a particular disease state, the invention would be valuable in the design of diagnostic assays to detect the presence of isozyme mRNA.

An abnormal level of nucleotide sequences encoding a PDE_XIV in a biological sample may reflect a chromosomal aberration, such as a nucleic acid deletion or mutation. Accordingly, nucleotide sequences encoding a PDE_XIV provide the basis for probes which can be used diagnostically to detect chromosomal aberrations such as deletions, mutations or chromosomal translocations in the gene encoding PDE. PDE_XIV gene expression may be altered in such disease states or there may be a chromosomal aberration present in the region of the gene encoding a PDE_XIV.

A PDE_XIV nucleic acid antisense molecule may be used to block the activity of the PDE_XIV in conditions where it would be preferable to elevate cyclic nucleotide levels.

The present invention also relates to the use of genetically engineered host cells expressing a PDE_XIV or variant, homologue, fragment or derivative thereof in screening methods for the identification of inhibitors and antagonists of the PDE_XIV that would modulate phosphodiesterase activity thereby modulating cyclic nucleotide levels. Such genetically engineered host cells could be used to screen peptide libraries or organic molecules capable of modulating PDE_XIV activity. Antagonists and inhibitors of PDE_XIV, such as antibodies, peptides or small organic molecules will provide the basis for pharmaceutical compositions for the treatment of diseases associated with, for example, CNS disorder, cardiovascular disorder, GI disorders, cancer, male erectile dysfunction. Such inhibitors or antagonists can be administered alone or in combination with other therapeutics for the treatment of such diseases.

The present invention also relates to expression vectors and host cells comprising polynucleotide sequences encoding PDE_XIV or variant, homologue, fragment or derivative thereof for the *in vivo* or *in vitro* production of PDE_XIV protein or to screen for agents that can affect PDE_XIV expression or activity.

Additionally, the present invention relates to the use of a PDE_XIV polypeptide, or variant, homologue, fragment or derivative thereof, to produce anti-PDE_XIV antibodies which can, for example, be used diagnostically to detect and quantitate PDE_XIV levels in disease states.

The present invention also relates to pharmaceutical compositions comprising effective amounts of inhibitors or antagonists of PDE_XIV protein (including anti-sense nucleic acid sequences) in admixture with a pharmaceutically acceptable diluent, carrier, excipient or adjuvant (including combinations thereof).

The present invention relates to pharmaceutical compositions which may comprise all or portions of PDE_XIV polynucleotide sequences, PDE_XIV antisense molecules, PDE_XIV polypeptides, protein, peptide or organic modulators of PDE_XIV bioactivity, such as inhibitors, antagonists (including antibodies) or agonists, alone or in combination with at least one other agent, such as stabilizing compound, and may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water.

The present invention further provides diagnostic assays and kits for the detection of PDE_XIV in cells and tissues comprising a purified PDE_XIV which may be used as a positive control, and anti-PDE_XIV antibodies. Such antibodies may be used in solution-based, membrane-based, or tissue-based technologies to detect any disease state or condition related to the expression of PDE_XIV protein or expression of deletions or a variant, homologue, fragment or derivative thereof.

The following samples were deposited in accordance with the Budapest Treaty at the recognised depositary The National Collections of Industrial and Marine Bacteria Limited (NCIMB) at 23 St. Machar Drive, Aberdeen, Scotland, United Kingdom, AB2 1RY on 18 December 1998:

E. coli pHS-PDE_XIV	NCIMB number NCIMB 40995
E. coli pMM-PDE_XIV	NCIMB number NCIMB 40996

The present invention also encompasses sequences derivable and/or expressable from those deposits and embodiments comprising the same.

The present invention will now be described by way of example, in which reference is made to the following Figures:

Figure 1 which presents a photographic image

Figure 2 which presents a photographic image;

Figure 3 which presents a Table and a photographic image;

Figure 4 which presents an alignment of nucleotide sequences;

Figure 5 which presents an alignment of protein sequences; and

Figure 6 which presents a graph.

Northern Hybridisation and Probe preparation

Northern blots, obtained from Clontech, were prehybridised for 1 hour in ExpressHyb hybridisation solution (Clontech) at 55°C before a radiolabelled new PDE fragment (DNA was labelled using the Megaprime random labelling system (Amersham) strictly following the manufacturers instructions with 50uCi of ^{32}P-dATP) was added to fresh Expresshyb and hybridised to the blot overnight at 55°C, with gentle shaking. Blots were then washed 3X at room temperature for 10 minutes each in 2XSSC (150 mM NaCl, 30 mM Na.citrate) followed by 2 washes in 0.2XSSC (15 mM NaCl, 3 mM Na.citrate) at 55°c for 20 minutes each. Blots were then exposed to autoradiographic film.

Polymerase Chain Reactions (PCR)

PCRs were performed using standard reagents and conditions. Briefly, all reaction buffers and enzymes were obtained in kit format from either Clontech (for rapid amplification of cDNA ends (RACE) reactions) or from Life technologies for standard PCR. Oligonucleotides were obtained from a commercial supplier (OSWEL DNA services) and used at a concentration of 400nM. Reactions were performed on a MJ Research PTC-200 thermal cycler, using cycling parameters as recommended by the manufacturer of the kit being used.

The identification of new PDE.

The clone (IMAGE clone id 1364394) was obtained from Research Genetics (2130, Memorial Pkwy, SW Huntsville, AL 358801, USA) as a stab culture in L-agar. Bacteria from the stab culture were streaked onto a 37 mm L-agar plate in the presence of ampicillin at a concentration of 100mg/ml. After overnight growth at 37°C a single clone was picked, using sterile technique, into 5ml of LB-broth containing ampicillin at a concentration of 100 mg/ml and grown at 37°C overnight with shaking (220 rpm). Plasmid DNA was isolated from the bacteria using a standard commercially available miniprep kit (Qiagen™) and strictly following the manufacturers instructions. The isolated DNA was then subjected to full length sequencing, using standard proprietary kits and reagents and an ABI (PE Applied Biosystems Incorporated) automated sequencer.

Isolation of a putative full length cDNA clone for PDE_XIV

To facilitate the isolation of a murine full length cDNA for PDE_XIV containing the full coding region and a terminator codon, the expression of new PDE was determined in a range of tissues using Northern hybridisation. These data (Figures 1 and 2) show that whilst the messenger RNA (length 5.5kb), which hybridises to new PDE, is present in several tissues, it is particularly highly expressed in murine brain, skeletal muscle & liver and at the following stages of embryological development, 10.5, 13.5, 15.5 days. The 13.5 day murine embryonic cDNA library was used in a cDNA positive selection method (GeneTrapper, Life Technologies, Inc.) to isolate the PDE_XIV. The method was carried out as manufacturers instructions. The original EST sequence of clone 1364394 was in the reverse orientation, therefore, design of the selection oligonucleotide needed to be identical to the coding strand running 5' to 3', this step was critical to the success of the cloning strategy. The oligonucleotide used for the selection and repair is detailed below.

Oligo 1:

Select1 5'-GGT CAC AGA ACT GCC ACT ATG GTT AAA TGT- 3'

To isolate the human homologue of the murine PDE_XIV, PCR primers were designed based on the known EST 1364394 sequence, (Primer 1 and Primer 2) and used to screen a panel of cDNA libraries. PCR's carried out using these primers (the PCR was set up using reagents obtained from a PCR Reagent System kit (Life Technologies) and following the manufacturers instructions, with standard cycling parameters) resulted in the generation of a fragment of the expected size, 164bp, obtained from the human HELA cell line cDNA library(Life Technologies, Inc.), using the same selection/repair oligonucleotide (Oligo 1) the human clone was isolated.

Primer 1:

newPDE1 5' -ACC GCT CAG AGA TTT CAC AGC A - 3'

Primer 2:

newPDE2 5' -CCC GTC TGA CCC CTT AGT CGT A -3'

Clones were selected for follow up through screening by colony PCR using the above primers and the following method. Using a sterile toothpick a single colony was picked and added to a tube containing 25ul of 1*PCR supermix (Life Technologies, Inc) & 200nM of primers. The tube was vortex'ed for 1 second, centrifuged for 2 seconds at 14,000 x g. The tubes were then placed in a pre-warmed thermal cycler (MJ Research PTC-200 thermal cycler) 94°C. PCR was performed using the following program: 1 cycle; 94°C, 1 minute, followed by 30 cylces of: 94°C, 30 seconds, 55°C, 30 seconds, 72°C, 1 minute. PCR products were then analysed by gel electrophoresis using standard methods.

Analysis of the isolated murine clone allowed the assembly of a contiguous sequence of 2823 bp which contains an ORF of 446 residues. The full length cDNA sequence for murine PDE_XIV is presented as SEQ ID No. 5 with the translation of the largest open reading frame within this cDNA presented as SEQ ID No. 1. The coding region is presented as SEQ ID No. 3.

Analysis of the isolated human clone allowed the assembly of a contiguous sequence of 2992 bp which contains an ORF of 288 residues. The cDNA sequence for human PDE_XIV is presented as SEQ ID No. 6 with the translation of the largest open reading frame within this cDNA presented as SEQ ID No. 2. The coding region is presented as SEQ ID No. 4. This clone has been used to screen a master RNA blot (Clontech) using standard Northern bolt methods to identify the tissue distribution for this cDNA. The results (see Figure 3) show that the transcript is highly expressed in the putamen and Caudate nucleus of the brain, in addition to Occipital lobe of the brain, heart, ovary, pituitary gland, kidney, liver, small intestine, thymus, and appendix.

Like all mammalian phosphodiesterases sequenced to date PDE_XIV contains a conserved catalytic domain sequence of approximately 250 amino acids in the carboxyl-terminal half of the protein that is thought to be essential for catalytic activity. This segment comprises amino acids 108 to 413 in SEQ ID 1 and exhibits sequence conservation with the corresponding region of other PDE's. From the nucleotide alignment (see Figure 4) and the protein alignment (see Figure 5) it is clear to see that the human PDE_XIV clone is truncated at the 3' end due to a premature stop codon at position 1102bp.

Phosphodiesterase assays on crude mammalian cell lysates

Both the murine & human PDE_XIV cDNA clones were transfected into mammalian COS7 cell line (ATCC) using Lipofectamine (Life Technologies, Inc) and standard methods. The COS7 cells were harvested 72 hr's post transfection and assayed for PDE activity using the following phosphodiesterase activity, a commercially available SPA (scintillation proximity assay) kit (Amersham) for cAMP SPA assay (Amersham). Serial

dilution's of the cell lysates were made in lysis buffer (50 mM HEPES pH 7.2, 1.92 mM $MgCl_2$, 50 mM KCL, 10 mM EGTA, 1 protease inhibitor tablet/50mls) to dilute out any endogenous inhibitory effects. To 25 ml of diluted crude lysate, 25 ml of buffer D (Buffer C + BSA @ 2mg/ml) was added followed by 50 ml of buffer C (20 mM Tris.HCL (pH 7.4), 5 mM $MgCl_2.6H_20$) containing 500 nM of cAMP substrate, (20 µl of [^{3}H] cAMP (1 µM. 4 µCi/ml) plus 423 µl of cold cAMP, 10 µM/5ml). The reaction was incubated for 30 minutes at 30°C. 50 µl of SPA beads was then added, immediately centrifuged at 2000 rpm for 5 miniutes. Reading were collected on a Topcount (Wallac) scintillation counter.

The results (see Figure 6) illustrate that both the human and murine PDE_XIV clones to be active, 10 fold higher than the mock transfected control.

All publications and patents mentioned in the above specification are herein incorporated by reference.

Various modifications and variations of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

SEQUENCE LISTINGS

The following sequences are presented:

SEQ ID No. 1 = murine amino acid sequence

SEQ ID No. 2 = human amino acid sequence

SEQ ID No. 3 = murine coding nucleotide sequence

SEQ ID No. 4 = human coding nucleotide sequence

SEQ ID No. 5 = murine cDNA sequence

SEQ ID No. 6 = human cDNA sequence

SEQ ID No. 1

```
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SHGLIHHFKLDMVTLHRFLVMVQEDYHGHNPYHNAVHAADVTQAMHCYLKEPKLASFLTPLDIMLGLLAA 210
AAHDVDHPGVNQPFLIKTNHHLANLYQNMSVLENHHWRSTIGMLRESRLLAHLPKEMTQDIEQQLGSLIL 280
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HRRRGSGQDLAGPAPETLEQTEGATP 446
```

SEQ ID No. 2

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LTNGNSLV 288
```

SEQ ID No. 3

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ATACAAATTGGTTTCATGACTTACATCGTGGAGCCGCTGTTCCGGGAGTGGGCCCGGTTTACTGGGAACA
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TCAGCACAGACGCAGGGGCAGCGGCCAGGACCTGGCGGGCCCCGCACCTGAGACCCTGGAGCAGACAGAA
GGTGCCACGCCCTAA
stop

SEQ ID No. 4

start
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stop

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SEQ ID No. 5

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SEQ ID No. 6

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TCATAGCTCTCTCCCTATAGTGAGTCGTATTATAAGCTAGGCACTGGCCGTC 2992
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CLAIMS

1. An amino acid sequence comprising the sequence presented as Formula I or a variant, homologue, fragment or derivative thereof.

2. An amino acid sequence comprising the sequence presented as Formula I.

3. A nucleotide sequence encoding the amino acid sequence as defined in claim 1 or claim 2.

4. A nucleotide sequence comprising the sequence presented as SEQ ID No. 3 or SEQ ID No. 4 or a variant, homologue, fragment or derivative thereof.

5. A nucleotide sequence comprising the sequence presented as SEQ ID No. 3 or SED ID No. 4.

6. A nucleotide sequence that is capable of hybridising to the nucleotide sequence according to any one of claims 3 to 5.

7. A nucleotide sequence that is capable of hybridising to the nucleotide sequence according to claim 6.

8. A vector comprising the nucleotide sequence according to any one of claims 3 to 7.

9. A host cell into which has been incorporated the nucleotide sequence according to any one of claims 3 to 7.

10. An assay method for identifying an agent that can affect PDE_XIV activity or expression, the assay method comprising

contacting an agent with an amino acid according to claim 1 or claim 2 or a nucleotide sequence according to any one of claims 3 to 7; and

measuring the activity or expression of PDE_XIV;

wherein a difference between a) PDE activity or expression in the absence of the agent and b) PDE activity or expression in the presence of the agent is indicative that the agent can affect PDE_XIV activity or expression.

11. An assay method according to claim 10 wherein the assay is to screen for agents useful in the treatment of any one or more of a CNS disorder, a cardiovascular disorder, a GI disorder, cancer, a sexual dysfunction.

12. A process comprising the steps of:

(a) performing the assay according to claim 10 or claim 11;

(b) identifying one or more agents that do affect PDE_XIV activity or expression; and

(c) preparing a quantity of those one or more identified agents.

13. A method of affecting *in vivo* PDE_XIV activity or expression with an agent;

wherein the agent is capable of affecting PDE_XIV activity or expression in an *in vitro* assay method;

wherein the *in vitro* assay method is the assay method defined in claim 10 or claim 11.

14. Use of an agent in the preparation of a pharmaceutical composition for the treatment of a disease or condition associated with PDE_XIV, the agent is capable of having an effect on the activity or expression of PDE when assayed *in vitro* by the assay method according to claim 10 or claim 11.

15. An enzyme capable of having an immunological reaction with an antibody raised against PDE_XIV.

16. A nucleotide sequence coding for a PDE, wherein the nucleotide sequence is obtainable from NCIMB 40995 or NCIMB 40996.

17. A PDE wherein the PDE is expressable from a nucleotide sequence obtainable from NCIMB 40995 or NCIMB 40996.

18. Use of an agent which has an effect on the activity of PDE_XIV or the expression thereof in the preparation of a pharmaceutical composition for the treatment of a disease or condition associated with PDE_XIV.

19. A recombinant PDE_XIV enzyme.

20. A recombinant nucleotide sequence encoding a PDE_XIV enzyme.

21. A PDE_XIV enzyme substantially as described herein.

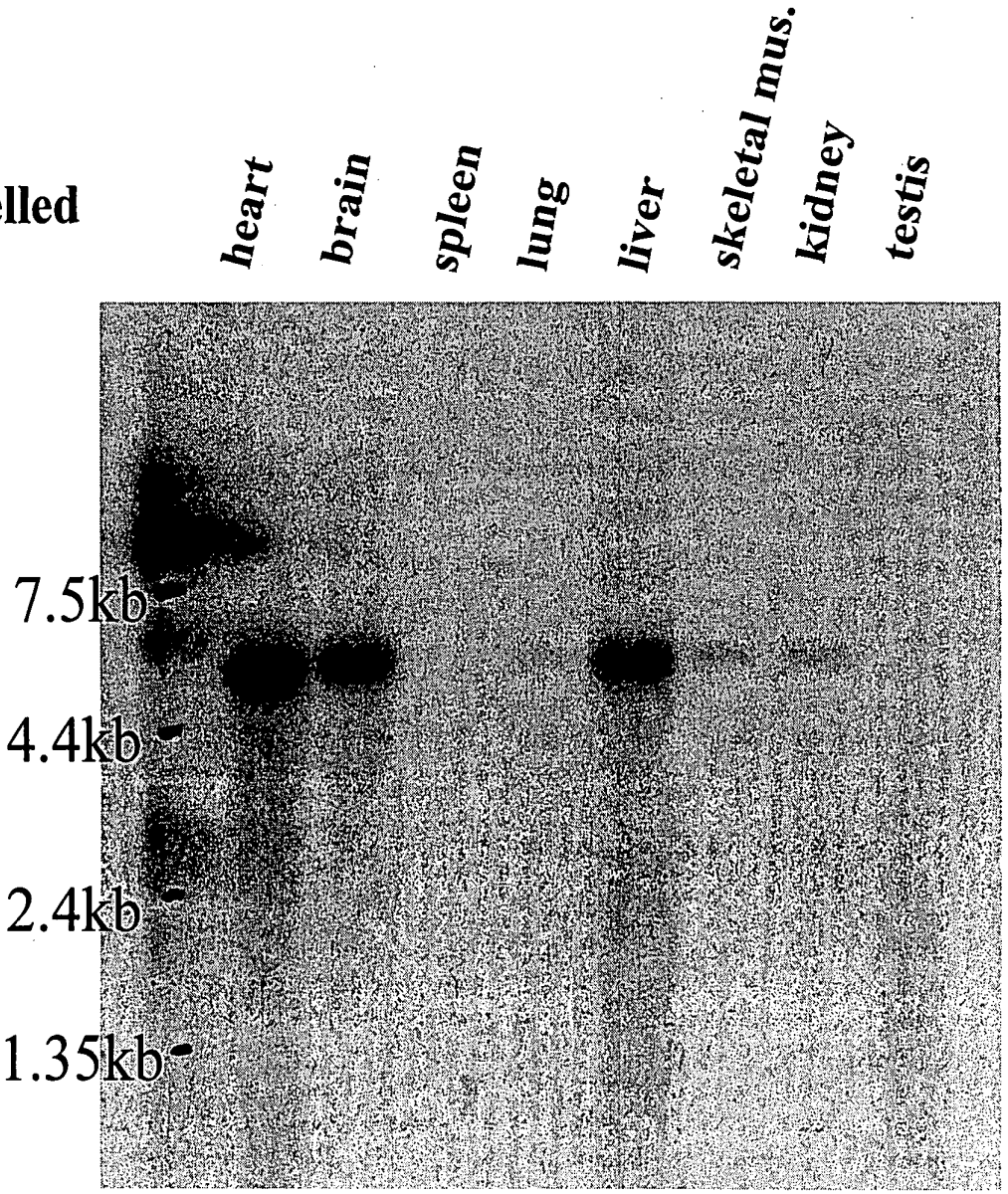
ABSTRACT

ENZYME

Amino acid sequences and nucleotide sequences are described. The amino acid sequences comprise the sequence presented as SEQ ID No. 1 or SEQ ID No. 2 or a variant, homologue, fragment or derivative thereof.

Fig 1

Murine MTN Blot probed with ^{32}P-labelled murine PDE_XIV



1/9

Fig 2

Murine Embryo MTN Blot probed with ^{32}P-labelled murine PDE_XIV

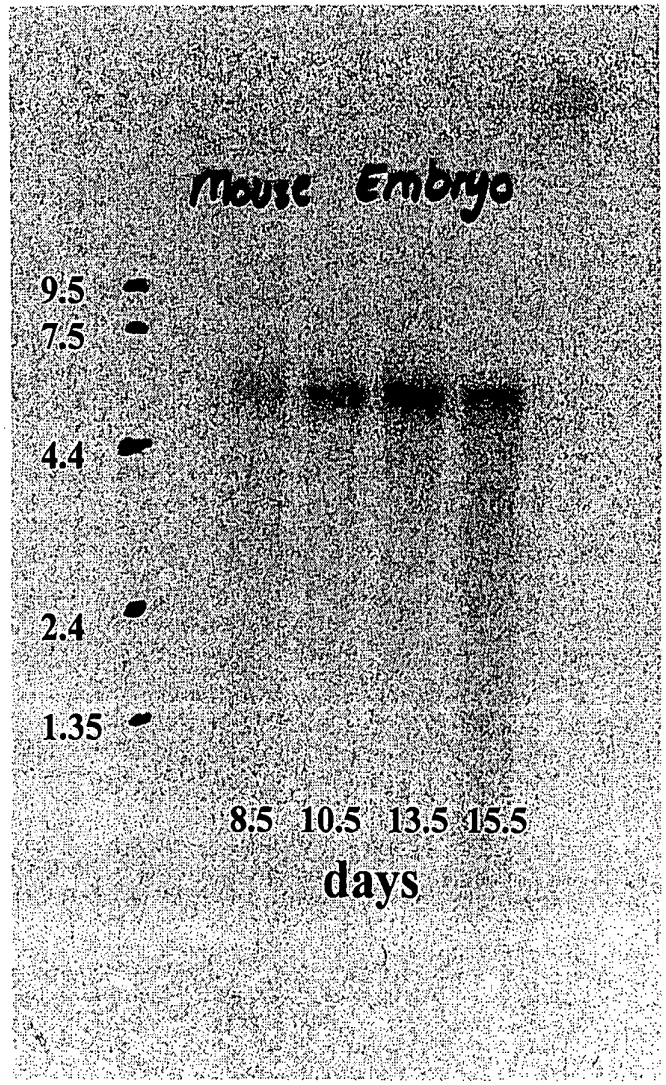


Figure 3

Human RNA Master Blot probed with ^{32}P-labelled human PDE_XIV

	1	2	3	4	5	6	7	8
A	whole brain	amygdala	caudate nucleus	cere-bellum	cerebral cortex	frontal lobe	hippo-campus	medulla oblongata
B	occipital lobe	putamen	substantia nigra	temporal lobe	thalamus	nucleus accumbeus	spinal cord	
C	heart	aorta	skeletal muscle	colon	bladder	uterus	prostate	stomach
D	testis	ovary	pancreas	pituitary gland	adrenal gland	thyroid gland	salivary gland	mammary gland
E	kidney	liver	small intestine	spleen	thymus	peripheral leukocyte	lymph node	bone marrow
F	appendix	lung	trachea	placenta				
G	fetal brain	fetal heart	fetal kidney	fetal liver	fetal spleen	fetal thymus	fetal lung	
H	yeast total RNA 100 ng	yeast tRNA 100 ng	*E. coli* rRNA 100 ng	*E. coli* DNA 100 ng	Poly r(A) 100 ng	human C_0t 1 DNA 100 ng	human DNA 100 ng	human DNA 500 ng

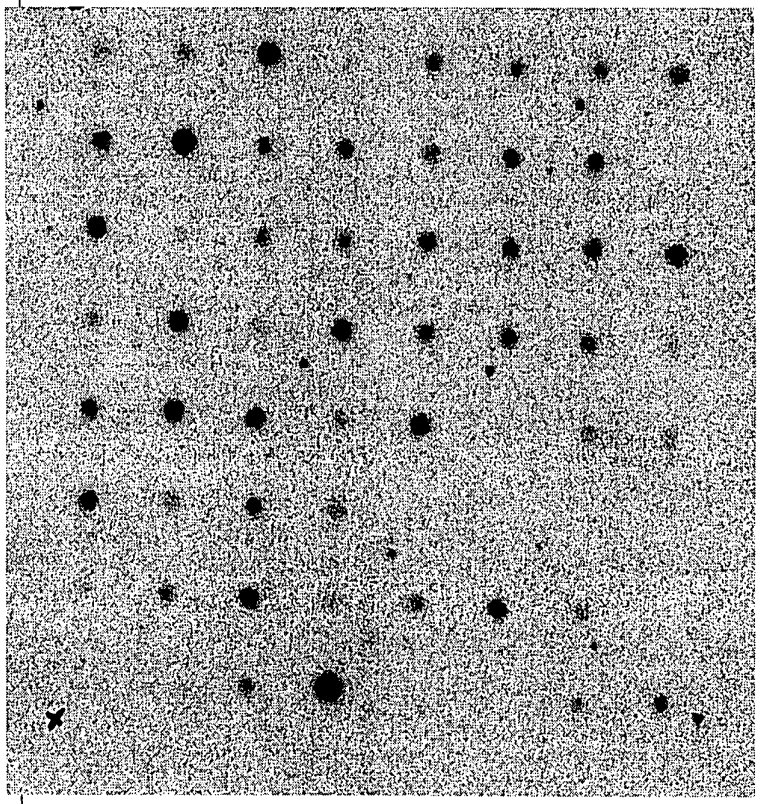


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Fig. 4. Alignment of the Murine and Human PDE_XIV nucleotide sequence

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Pileup: Genetics Computer Group.
   MSF: 3134  Type: N    Check:  5422   ..

 Name: mpdea_ oo  Len: 3134  Check:  5084  Weight:  0.001
 Name: hspdea_ oo  Len: 3134  Check:  338  Weight:  0.100

new mpde_       1 AGGTACGCCT GCAGGTACCG GTCCGGAATT CCCGGGTCGA CCCACGCGTC
new hspde_        ..........  ..........  ..........  ..........  ..........

new mpde_      51 CGGCCAGCCT CCCAGGCCGG CTGCCTGCTC ACCCAGCCAG TCGCTAGCTC
new hspde_      1 ..........  ..........  ..........  ..........  .CGGAAT.TC

mpdea_        101 TGGGCACTGC AGCAGGCTCG GCTCTGTCCC AGCGCTCGCT TGCTTGCTCG
hspde_          9 GATGCACTGC AGCAGGCTCG GCTCTGTCCC AGC....A..  ..........

mpdea_        151 CTCGCTCGGC TGGGAGAAAA GTGGTGTC.C TCGCCCAG..  AGAGCCTCTC
hspde_         43 ....CTTGTC TGGGAGAAAA GTGGTGTTAC TCACCCAGGG  AGAGTCTCTC

mpdea_        198 TCTC..CCTT CCTTCTTTCT CGAGCTCTCT GAGTCCTTTG GCGTTTCTTT
hspde_         89 TTTCTACCTT CCTTCTTTCT CGATCTCCTT GTGTGCTTTT GTGTTTCTTT

mpdea_        246 CTTTCTTTCC TTTTTTTTTT TTTTTTAATA TTTTCTTTTT CTTTCTATAA
hspde_        139 ATTTCTTTTC CTTTTTTTTC TT........  TTTTTTTTTT GTTACT....

mpde_         296 AACTTGCATA ATTATACTGC TAATCCTGGA TGAGGTTGCT GGATTCTGCA
hspde_        177 .........TA ATTATATTCC TAATCCTGGA TGAAGTTGCT GGATTCTGCA

mpde_         346 GCACAAATCT TCATGAACAA GCCGCACCGC TCAGAGATTT CACAGCATTC
hspde_        219 GCACAAGTCT TCATGAACAA GCAGCACCGC TCAGAGATTT CACGGCATTC

                                                    start codon
mpde_         396 AAAGGTCACA GAACTGCCAC TATGGTTAAA TGTCTTGTTT AATGGTTGAG
hspde_        269 AAAGGTCACA GAACTGCCAC TATGGTTAAA TGTCTTGTTT AATGGTTGAG

mpdea_        446 AGGTGTGGCG AAGTCTTGTT TGAGAGCCCT GAACAGAGTG TCAAATGTGT
hspde_        319 AGGTGTGGCG AAATCTTGTT TGAGAACCCC GATCAGAATG CCAAATGTGT

mpde_         496 TTGCATGCTA GGAGATGTAC GACTAAGGGG TCAGACGGGG GTTCCTGCCG
hspde_        369 TTGCATGCTG GGAGATATAC GACTAAGGGG TCAGACGGGG GTTCGTGCTG

mpde_         546 AACGCCGTGG CTCCTACCCA TTCATTGACT TCCGTCTACT TAACAATACA
hspde_        419 AACGCCGTGG CTCCTACCCA TTCATTGACT TCCGCCTACT TAACAGTACA

mpde_         596 ACACACTCAG GGGAAATTGG CACCAAGAAA AAGGTGAAAC GACTGTTAAG
hspde_        469 ACATACTCAG GGGAGATTGG CACCAAGAAA AAGGTGAAAA GACTATTAAG

mpde_         646 TTTCCAAAGA TACTTCCATG CATCTAGGCT TCTCCGGGGG ATTATACCGC
hspde_        519 CTTTCAAAGA TACTTCCATG CATCAAGGCT GCTTCGTGGA ATTATACCAC

mpde_         696 AGGCCCCTCT CCACCTGCTG GATGAAGACT ACCTTGGACA AGCAAGGCAC
hspde_        569 AAGCCCCTCT GCACCTGCTG GATGAAGACT ACCTTGGACA AGCAAGGCAT

mpde_         746 ATGCTCTCCA AAGTTGGAAC GTGGGACTTT GACATTTTCT TGTTTGATCG
hspde_        619 ATGCTCTCCA AAGTGGGAAT GTGGGATTTT GACATTTTCT TGTTTGATCG
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mpde_   796 CTTGACAAAT GGGAACAGTC TGGTAACTCT GTTGTGTCAC CTCTTCAACT
hspde_  669 CTTGACAAAT GGAAACAGCC TGGTAACACT GTTGTGCCAC CTCTTCAATA

mpde_   846 CCCATGGGCT CATCCACCAT TTCAAGCTCG ATATGGTGAC CTTGCACAGG
hspde_  719 CCCATGGACT CATTCACCAT TTCAAGTTAG ATATGGTGAC CTTACACCGA

mpde_   896 TTTCTGGTTA TGGTTCAGGA AGATTACCAC GGTCACAACC CATACCACAA
hspde_  769 TTTTTAGTCA TGGTTCAAGA AGATTACCAC AGCCAAAACC CGTATCACAA

mpde_   946 TGCTGTTCAC GCAGCCGACG TCACCCAGGC CATGCACTGT TACCTGAAGG
hspde_  819 TGCTGTTCAC GCAGCCGACG TCACCCAGGC CATGCACTGC TACCTGAAAG

mpde_   996 AGCCAAAGTT GGCAAGCTTC CTCACACCTC TGGACATCAT GCTTGGACTA
hspde_  869 AGCCAAAGCT TGCCAGCTTC CTCACGCCTC TGGACATCAT GCTTGGACTG

mpde_  1046 CTGGCTGCAG CAGCTCATGA CGTGGACCAC CCAGGGGTCA ACCAGCCATT
hspde_  919 CTGGCTGCAG CAGCACACGA TGTGGACCAC CCAGGGGTGA ACCAGCCATT

mpde_  1096 TTTGATCAAA ACTAACCACC ATCTTGCCAA CCTGTATCAG AATATGTCTG
hspde_  969 TTTGATAAAA ACTAACCACC ATCTTGCAAA CCTATATCAG AATATGTCTG

mpde_  1146 TACTGGAGAA TCACCACTGG CGATCTACAA TTGGCATGCT TCGAGAATCA
hspde_ 1019 TGCTGGAGAA TCATCACTGG CGATCTACAA TTGGCATGCT TCGAGAATCA

mpde_  1196 CGGCTCCTGG CTCACTTGCC AAAGGAAATG ACACAGG... .....ATATC
hspde_ 1069 AGGCTTCTTG CTCATTTGCC AAAGGAAATG ACGTAAGTGC TGCCGAGATG
                                            stop codon

mpde_  1238 GAACA..... ....GCA... ......GCTG GGCTCCCTCA TCTTGGCCAC
hspde_ 1119 AAACATACTG ATGTGCATGC AGTAAAGATA AGCCACTTTC TCTAGGGCA.

mpde_  1270 GGATATCAAC AGACAGAATG AGTTTCTGA. ......CCCG CTTAAAAGCT
hspde_ 1168 GGCTTGGGAC CTTTTGCGTG AATGGCAGAG AGCCCCCCGG CTGTACTTCC

mpde_  1313 CACCTCCACA ATAAAGATT. TGAGAC.... ..TGGAGAAT GT.ACAGGA.
hspde_ 1218 TGCCTGCACT GAGCTGTCTA TCAGAGGAGA TTTGGTGTCA GTTACAGCAA

mpde_  1354 ..CAGACACT TTATGCT.TC AGATCGCCTT GAAGTGTGCT GACATTTGCA
hspde_ 1268 CCCAGAAACC AAAATCTCTC TGTGTGCTTT GAAAGGGCCT TGCAGAGTCA

mpde_  1401 AT..CCTT.. GTC.GTATCT GGGAGATGAG CAAGCAGT.. GGAGTGAAAG
hspde_ 1318 ATGACCTACA GTCAGGAAAA GGGATAATAA ACAGCTCTCA GTTTTCACAC

mpde_  1444 GGT..... CTGTGAGGAA TTCTACAGAC AAGGTGACCT TGAAC..AG.
hspde_ 1368 GCTTCAGTAT CAGTGCTCAA CTTTGCCAAA TTCCCGACCT TTAGTTTAGC

mpde_  1484 AAGTTTGAAC TGGAAATCAG .TCCTCTTTG TAATCAAC.A GAAAGATTCA
hspde_ 1418 AAAATTGTCC TTCCATGTAG CTCCAAATAG TAAATATTTA TCAAGAAGGA

mpde_  1532 ATCCCTAGCA TACAAA...T TGGTTTCATG ACT.TACATC GTGGAGCCGC
hspde_ 1468 A.CCCAGGCA TTCTAAAGCT AGAGTTCAAA AAAGTATATT TTGTAATTGC

mpde_  1578 TGTTCCGGG. ...AGTGG.. GCCCGGTTTA CTGGG..AAC AGCACCCTGT
hspde_ 1517 TAGTCTCAGC AAAAATAGAA GTCAGAAATT CTTTTCTAAA ATGTCTTTTG

mpde_  1620 CGGAGAACAT GCTAAGCC.. ....ATCTCG CGCACAACAA AGCCCAGTGG
hspde_ 1567 CTAAGTAATT GAAATGGCCC TAGCATTTTT TTCACCAATT AATTTACCTT
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P5894.GB

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mpde_     1664 AAGAGCCT.G CTGTCCAATC AGCAC...AG ACGCA..... ...GGGGCAG
hspde_    1617 ACGTCTCTTG CACTTTAAAC AGAAGGGGAG ACACTCATTT TCTGGTTCAC

mpde_     1702 CG.......G CCAGGACCTG GCGG....GC CCCGC...AC CTGAGACCCT
hspde_    1667 TATTTGATAG CCATGGTATG TAGGCTGAGT CCCACTAAAT CTGAGGCCAT

                                                stop codon
mpde_     1738 GGAG.CAGAC AGAAGGTGCC ACGCCCTAAG GTAGCTGTC. .TGCTGA..T
hspde_    1717 TGTTTCATTT TCCTGGTG.. ..GCCCCAAG TTAGCTGCTA ATACTGTCTT

mpde_     1783 GCACGGCCA. .......TCT G.TCCGTCCA .....CAGGA GCACGGCC..
hspde_    1763 CCAAGGCCAC CATTAATTCT GATCTGTTTA ATGAACACGT GCAGAACCCA

mpde_     1817 ...ATCC... .........G TCC...GACT GC........ .CCTCGCAAC
hspde_    1813 AGAAACCTAG GTGAAAAGAG TACATAGATT GCTGTACCCT TCTTCAAGAC

mpde_     1840 AAGCCCATCA CGCTGGGTTT CGATGCCAT. .CCGCCTGCC A.CTTACC..
hspde_    1863 AAGCACATAA CTTGAGGTCA AGGACCAAGT GCTGTCTCCC AACTGAACAA

mpde_     1885 ...GCCTCCC TTCGTTGATC CAAGTGTACA AAAGCCATTG ...TCACCTC
hspde_    1913 GCAGTATACT CTGGGTTGTG GATTGATTCC TGGCCCTCTG ATTTGATCTC

mpde_     1929 AGCAT..... .TAGCTGCC. ..GAAATGGG CGGCTCTATC CCGTCATTGG
hspde_    1963 ATGCTGTTTC CTAGCACCCA GAGGAATGTG AAATTTGCAG GAGGAATTTC

mpde_     1970 AG..CTGAT. ...TCTGGGG CGGCTGCCCC AACCGAAAC. .........G
hspde_    2013 AGTTCTGATA AATTTTTACT CCCTGGAACT AAATAAAACC AGTTCTCGTG

mpde_     2004 CCTGGAAGTA AGAA..AGGG GTGCTTCTGC CGTGTTCGCC TCTGGCCCTT
hspde_    2063 CATGGAATAA AAACTTATGC CTCTTACTAG AATAATAAAT TGCAAAGATT

mpde_     2052 GGTCACGCTG ACTGGCAGTA GCTCCTAAGT CCAGAGCATT TTAACGTTTG
hspde_    2113 GAAAGAATTA AATGCAAAAA GAACTAAAAA CTAGAGCAAA AGATCAAGTG

mpde_     2102 CCATC..GGA CAGCTGACCT ...GCATGAC ACCAGCAT.. .ACTTGGAAC
hspde_    2163 AGAAGAAGAA AAGAGGAGGT AAGGAGAGAG ACAAGGAAGA AAGAAGGAGA

mpde_     2144 TGCAAAACTG GTCTTGCGTG CCAGAGCACA AACGAGAGTG TGAGAGAAA.
hspde_    2213 AGGAAAGGAA GAATAGTGAG G.ACAGGAAA GAAGAAAATG CAAGGGAAAT

mpde_     2193 ..GTACCTTC TATTT..TAA TAATAATTAT TATTATAAAA TA....ATAA
hspde_    2262 GGGAAAGGAC TCTGGGGTGA CCAGACTTCT CCTGGTCAGT ACCTGCATTC

mpde_     2235 ATCTTTTTAA CTTTT..ATA TTTCATGCAC CAGACAATGG GTCTAAAACT
hspde_    2312 ATCCTGTTTG TTACTCAATA TTTCTTTCCT AAAATATTCA TTTCACATCT

mpde_     2283 TTGGA...CA AGTAATACTC TGCGTACCCA AACCTAAGAG G......GGG
hspde_    2362 ATGGATTCCA ATGAAAAATA TATTTTTATG TGTCTTTGTG GAACACAGTG

mpde_     2324 TTC...ATTA TTTT.GCTAT T.GACTC... ..TATGCCAC ATTGGGTCCG
hspde_    2412 TTATAAATTG TTTTTGCCAG AAGAATAATT GTTATACAAT AATATATGTG

mpde_     2364 AGA..TGTGG CACCATTGCG ATTTCTGAAA CCACGCGTCC .CCTCCCATC
hspde_    2462 AAAACTTTAT TACAAAAGCC ATTATCATAA TCATTATTAT TCCTTCTATC

mpde_     2411 TGGTGGAAGG TGCTGTACAG CCCGTCCC.. ..TTTGCACC GTTAGCCAAT
hspde_    2512 ACA.GGTAAA TGCTTTAATG TCATTTTTCT GATTTTAAAA GTAGGGCAGG
```

```
mpde_    2457 CCGTCTTT..  ....TACGGA  ...TTCAGTG ACCTGTTTAT ATTCACAA.G
hspde_   2561 TTAATTGTAG AAAGTAAGGA AAATTCAGGA AAGTGTTAGT TTGAACTATG

mpde_    2497 TGTACATTTT CTGT..AAAT ACCAAACGCT ACTGA.....  ......TTCC
hspde_   2611 TGAAGTTGCT CTTTTTAAGG GCCAAAAACA GGAGACTTTT AGCACTTTCA

mpde_    2534 CATGC..CA.  ....AAATAC ACGAGTATTA TGGGATTGCT A.....CCTG
hspde_   2661 TATGTTTCAG CTTGATATGA AAGAGAAAAC TGAAACTGCT AGTAATCCTG

mpde_    2571 .........T ATAAACAATG GCACTGTGAA CAGAATA... .CTGTTAGTT
hspde_   2711 CCATCCAGGT ATAGTTCATG TTAACCTGGC TAGTTTATTT TCTTTTAGTC

mpde_    2608 TTAATACAAG AGAATGCATT TGTAAATATG GTATAGAGTT TATTAATATA
hspde_   2761 TTTTTTCAAT ACAAA.CTTA TTTTAACAAA ATAT.GATTA TATTTGGGGA

mpde_    2658 CTGTTGTTCG CAGATAAAGG CCTTAACTTT AAAAAAAAAA AAAAAAAAA.
hspde_   2809 ACTTATTTTA CAGTTTACGT CCTGAAATTT TTTATTTACA ATAAAGACTT

mpde_    2708 .......AAAA AAAAAAAAAA AAAAAGGGGC GGCCGCTCTA GAGGATCCCT
hspde_   2859 TTTTCCAAAT CAAAAAAAAA AAAAAAGGGC GGCCGCTCTA GAGGATCCCT

mpde_    2752 CGAGGGGCCC AAGCTTACGC GTGCATGCGA CGTCATAGCT CTCTCCCTAT
hspde_   2909 CGAGGGGCCC AAGCTTACGC GTGCATGCGA CGTCATAGCT CTCTCCCTAT

mpde_    2802 AGTGAGTCGT ATTATAAGCT AG........ .... 2823
hspde_   2959 AGTGAGTCGT ATTATAAGCT AGGCACTGGC CGTC 2992
```

Figure 5. Protien Alignment of the Murine & Human PDE_XIV

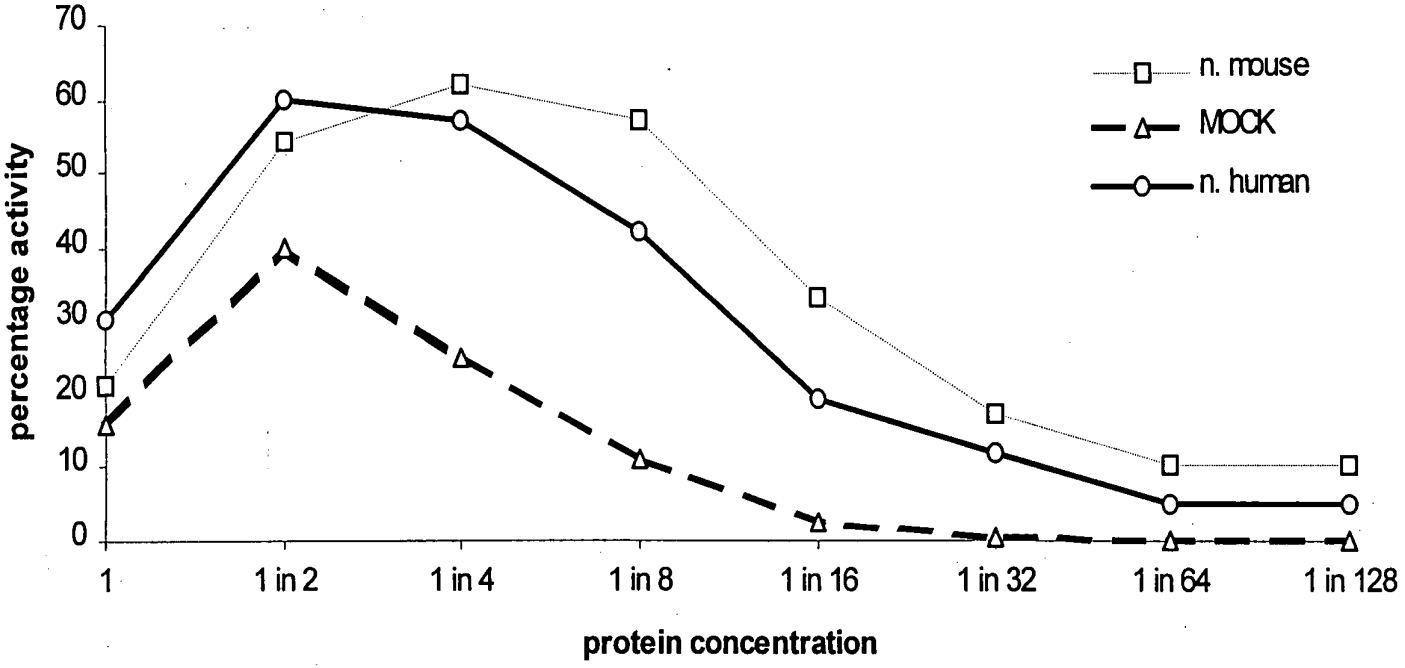
CLUSTAL W (1.7) multiple sequence alignment

```
mpde    MSCLMVERCGEVLFESPEQSVKCVCMLGDVRLRGQTGVPAERRGSYPFIDFRLLNNTTHS 60
hspde   MSCLMVERCGEILFENPDQNAKCVCMLGDIRLRGQTGVRAERRGSYPFIDFRLLNSTTYS 60
        ***********:***.*:*..********:******** ***************.**:*

mpde    GEIGTKKKVKRLLSFQRYFHASRLLRGIIPQAPLHLLDEDYLGQARHMLSKVGTWDFDIF 120
hspde   GEIGTKKKVKRLLSFQRYFHASRLLRGIIPQAPLHLLDEDYLGQARHMLSKVGMWDFDIF 120
        ************************************************** ******

mpde    LFDRLTNGNSLVTLLCHLFNSHGLIHHFKLDMVTLHRFLVMVQEDYHGHNPYHNAVHAAD 180
hspde   LFDRLTNGNSLVTLLCHLFNTHGLIHHFKLDMVTLHRFLVMVQEDYHSQNPYHNAVHAAD 180
        ********************:**************************.:***********

mpde    VTQAMHCYLKEPKLASFLTPLDIMLGLLAAAAHDVDHPGVNQPFLIKTNHHLANLYQNMS 240
hspde   VTQAMHCYLKEPKLASFLTPLDIMLGLLAAAAHDVDHPGVNQPFLIKTNHHLANLYQNMS 240
        ************************************************************

mpde    VLENHHWRSTIGMLRESRLLAHLPKEMTQDIEQQLGSLILATDINRQNEFLTRLKAHLHN 300
hspde   VLENHHWRSTIGMLRESRLLAHLPKEMT------------------------------ 268
        ****************************

mpde    KDLRLENVQDRHFMLQIALKCADICNPCRIWEMSKQWSERVCEEFYRQGDLEQKFELEIS 360
hspde   ------------------------------------------------------------

mpde    PLCNQQKDSIPSIQIGFMTYIVEPLFREWARFTGNSTLSENMLSHLAHNKAQWKSLLSNQ 420
hspde   ------------------------------------------------------------

mpde    HRRRGSGQDLAGPAPETLEQTEGATP 446
hspde   -------------------------
```

Zinc binding motif's are highlighted in bold.
Non-Catalytic domain in italics.

Figure 6.

SPA assay to determine the cAMP hydrolytic activity of murine and human PDE_XIV.



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